CLINICAL MICROBIOLOGY REVIEWS, July 1993, p. 266–310
0893-8512/93/030266-45$02.00/0

Vol. 6, No. 3

The *Mycobacterium avium* Complex

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CLASSIFICATION

Conventional Criteria

Mycobacteria have been conventionally classified into four or five broad taxonomic groups on the basis of the following general criteria: pathogenicity for humans and animals, rate of growth at optimum temperatures, and effect of visible light on pigment production. These criteria have proven useful for the classification of mycobacteria since first developed by Runyon and others in the early 1950s (179, 408, 451); recent evidence from comparative 16S rRNA sequencing studies (437) has corroborated their taxonomic validity. Accordingly, mycobacteria included in the *Mycobacterium avium* complex (MAC) are classified as acid-fast, slowly growing bacilli that may produce a yellow pigment in the absence of light (exposure to light often intensifies pigment production). The MAC is composed of opportunistic pathogens capable of causing disease in both animals (448) and humans (253, 504). Tuberculosis, caused by mycobacteria in the *M. tuberculosis* complex, has been traditionally viewed as the "typical" mycobacterial disease; thus, other species of mycobacteria (with the exception of *M. leprae*) have been viewed by contrast as "atypical." Consequently, mycobacteria other than *M. tuberculosis* and *M. leprae* have been commonly referred to by the imprecise and taxonomically inappropriate term atypical mycobacteria. Other terms commonly applied to these mycobacteria are mycobacteria other than tuberculosis, or MOTT, and nontuberculous mycobacteria (NTM). Wayne and Sramek (474) recently reviewed the systematics of the mycobacteria and pointed out that the distinction among *M. tuberculosis*, *M. leprae*, and the other species of mycobacteria is not the ability to cause serious disease in humans but rather differences in natural habitats and contagiousness. Thus, they proposed the term potentially pathogenic environmental mycobacteria, or PPEM, a term which emphasizes the importance of environmental exposure to these mycobacteria since there is little or no contagiousness between humans associated with these microorganisms.

Serotypes

The MAC is a serological complex of 28 serovars of two species, *M. avium* and *M. intracellulare*, which sometimes has been extended to include three additional serovars of a third species, *M. scrofulaceum*. Therefore, the mycobacteriology literature may include references to the complex as the *M. avium-M. intracellulare* complex or the *M. avium-M. intracellulare-M. scrofulaceum* intermediate complex. However, the inclusion of *M. scrofulaceum* is no longer appropriate given our current understanding of mycobacterial systematics (474). The distinction between *M. avium* and *M. intracellulare* is now well established, and Thorel et al. (450) have proposed three subspecies of *M. avium* on the basis of phenotypic properties and nucleic acid studies: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum*. The International Working Group on Mycobacterial Taxonomy has suggested, however, that there is taxonomic evidence for a third genospecies within the MAC (473).

Serovar distinctions within the MAC are based on a seroagglutination procedure originally described by Schaefer (419). Later, Brennan and coworkers (65–67) showed that the serovar antigens of the MAC have a common lipopeptidyl-*O*-methyl rhamnose linked to an oligosaccharide; i.e., serologic specificity was conferred by the specific oligosaccharide residues of the C-mycoside glycopeptidolipids (GPLs), which are integral constituents of the cell wall and envelope. On the basis of this more complete knowledge of the chemistry of the serovar antigens, strains now are serotyped by thin-layer chromatography (68, 454) and enzyme-linked immunosorbent assay (ELISA) analysis (498) of species- and type-specific glycolipids as well as by the conventional seroagglutination procedure. More recently, Rivoire et al. (399) described an ELISA system that used murine monoclonal antibodies to specific sugar epitopes of the MAC derived with either purified GPL antigens or synthetic neoantigens. The focus of the latter study was to generate monoclonal antibodies that were absolutely specific for each of the major serovars of the MAC. In achieving this objective, the oligosaccharide haptens were defined for the

most common serovars of the MAC isolated from patients with AIDS in the United States, i.e., serovars 1, 4, and 8 (399). Serovar and DNA relatedness studies have led to a consensus that serovars 1 through 6 and 8 through 11 are assigned to *M. avium* while serovars 7, 12 through 17, and 19, 20, and 25 are assigned to *M. intracellulare* (413).

Multilocus Enzyme Electrophoresis Types

Recently, Wasem et al. (470) examined 35 strains of the MAC and an additional 12 species or strains of other mycobacteria by multilocus enzyme electrophoretic typing, using 20 different enzymes. A total of 33 electrophoretic types (ETs) were identified, of which 24 types included the 35 MAC strains. Two distinct clusters were apparent in the resulting dendogram of the 24 ETs: an *M. intracellulare* cluster and an *M. avium* cluster. The clustering agreed entirely with the species identity as determined by the GenProbe nucleic acid hybridization system. When the analysis was extended to include all 33 ETs, again two distinct clusters were observed, but with an *M. scrofulaceum* strain joined to the *M. intracellulare* cluster and an *M. paratuberculosis* strain joined to the *M. avium* cluster. All but one of the serovars separated into the *M. intracellulare* and *M. avium* clusters when ET types were compared with serovar classification. The common serovars, serovars 1, 4, 8 to 10, 14, and 16, could be subdivided into two to four ETs. Although the authors pointed out that serovar and ET designations are not interchangeable, it was of interest that serovars 1 to 4 and 8 to 10 appeared in the *M. avium* ET cluster and serovars 12, 14, 16, and 19 appeared in the *M. intracellulare* cluster. These results are in virtually complete agreement with earlier DNA-DNA relatedness studies or GenProbe DNA-rRNA hybridization and serovar studies. There are two DNA relatedness groups that make up the *M. avium-M. intracellulare* complex (a third group includes *M. scrofulaceum*) (400), and DNA relatedness studies first performed by Baess (13) and later confirmed by Yoshimura and Graham (500) showed that serovars 1 to 6 and 8 to 11 were *M. avium* whereas serovar 7 and serovars 13 to 28 were *M. intracellulare*. More recently, Saito et al. (413) used the GenProbe DNA-rRNA hybridization system to analyze the species distribution of serovars and concluded that serovar 21 is most likely *M. avium* and serovars 7, 12 to 20, and 25 are *M. intracellulare*; serovars 22 to 24 and 26 to 28 were too disordered to assign a species epithet.

Phage Types

Although phage typing has proven to be a useful tool for discriminating between strains of *M. tuberculosis* (434), there has been only a limited application of phage typing to the epidemiology of the MAC. Crawford et al. (111) described a technique of phage typing for the *M. avium-M. intracellulare-M. scrofulaceum* complex and applied the technique in a study of several hundred *M. avium-M. intracellulare-M. scrofulaceum* complex strains isolated from the environment, animals, and clinical specimens from geographically disperse humans (107). Only approximately one-third of the isolates and none of the environmental isolates were susceptible to the mycobacteriophages tested. Nevertheless, for susceptible strains, the phage-typing system appeared to be a reliable epidemiological tool, but the lack of phage susceptibility of the majority of strains is an important limitation. Crawford and Bates (107) pointed out that several factors can influence the susceptibility of mycobacteria to phage infection, including a requirement for accessible cell surface receptors, lysogenic immunity, the presence of a restriction-modification system, and plasmid interference. It is conceivable that all or any combination of these factors might influence the phage susceptibility of the MAC. Restriction-modification systems have been described in the MAC (110), and many MAC isolates carry plasmids (332). The lack of phage susceptibility may be an important complication to the otherwise exciting potential application of luciferase-phagemid systems to the direct detection and identification of the MAC in clinical specimens as well as susceptibility testing (15).

Plasmid Types

Plasmid typing may be similarly limited for epidemiology studies in that only 50% of clinical isolates and only 20% of environmental isolates carry plasmids (332); however, there is evidence that there may be an epidemiologically significant uneven distribution of MAC strains, both clinical and environmental, which carry plasmids. In a study of 26 MAC isolates from AIDS patients, Crawford and Bates (108) described three types of plasmids that were present in various configurations in all strains. Indeed, all strains carried plasmids that hybridized to recombinant molecules carrying fragments of a small plasmid (pLR7) derived from a serotype 4 strain of the MAC. However, this observation is somewhat at odds with other more recent studies that showed that only 5 of 16 MAC isolates from AIDS patients in Denmark carried plasmids (260) and that there was no difference in the rate of plasmid carriage in 128 strains from AIDS and non-AIDS patients in the United Kingdom (215). Morris et al. (344) determined the plasmid profiles of 12 separate *M. avium* isolates and identified multiple plasmids of <100 kb in 9 of 12 isolates. Although the pLR7 plasmid probe hybridized to DNA extracts from all plasmid-bearing strains, restriction analysis suggested that the plasmids were not identical. Morris et al. (344) concluded that plasmids may not be required for the development of disseminated MAC disease and the role of plasmids can be determined only by virulence transformation experiments. Meissner and Falkinham (332) showed that although on average only 19% of environmental isolates carried plasmids, 75% of isolates from aerosols carried plasmids. Also, the study by Hellyer et al. (215) concluded that plasmids were common in serovar 4 and 8 strains of the MAC and corroborated the observation of Crawford et al. (108) that these plasmids had DNA sequences homologous to that of the pLR7 plasmid. The role of plasmids in the biology and pathogenicity of the MAC may be important because of the association of plasmids with virulence factors (162, 382) and, in two studies, with antibiotic resistance (155, 339).

Large RFLP Types

Distinctions between MAC strains have been achieved by restriction fragment length polymorphism (RFLP) analysis of genomic DNA, using endonucleases with both frequent and infrequent restriction sites and separation of large DNA fragments. The application of the latter technique to mycobacteria takes into consideration that mycobacterial DNA contains a high percentage of guanine plus cytosine (62 to 70 mol%); therefore, restriction endonucleases with 6-base recognition sites that are rich in adenine and thymine are likely to cleave mycobacterial DNA into 30 or fewer fragments. The number of fragments can be predicted by a

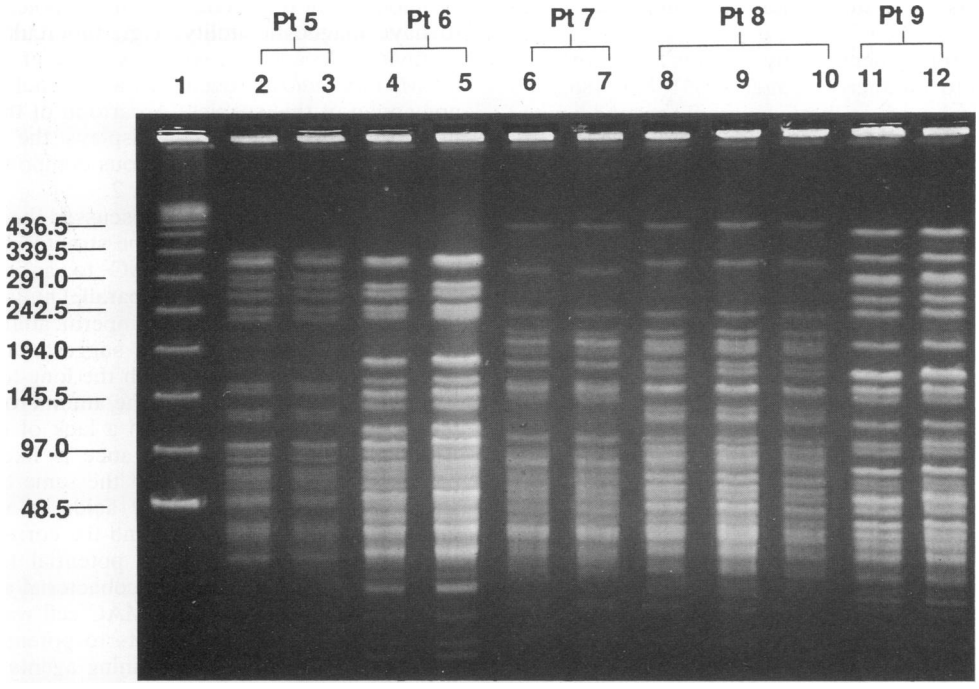

FIG. 1. Large RFLPs of two separate isolates of MAC from each of five patients, taken from Mazurek et al. (320). Mycobacterial DNA was restricted with *Xba*I, and fragments were separated by pulsed-field gel electrophoresis. For patients 6, 7, and 9, both isolates were from sputum, while for patients 5 and 8, the isolates were from different body sites. Reprinted with permission of the publisher.

nearest-neighbor analysis; however, as with other bacteria, in the few studies of the MAC that have been published, fewer fragments are generated than are predicted by such an analysis. Nevertheless, the number and size of fragments often approximate the total DNA content of the cell, and the resulting RFLP patterns most likely reflect the distribution of restriction sites within nearly the entire genome. Two endonucleases (with the corresponding restriction sites) that have proven useful in generating RFLP patterns with mycobacterial DNA are *Dra*I (AAATTT) and *Ssp*I (AATATT). These enzymes generate large restriction fragments that can be resolved only by field inversion gel electrophoresis or pulsed-field gel electrophoresis into fragments that range from 45 to >400 kb. Levy-Frebault et al. (299) examined various strains of mycobacteria by RFLP analysis by using *Dra*I and pulsed-field gel electrophoresis and showed that strains of *M. paratuberculosis* were identical to strains of mycobacteria isolated from patients with Crohn's disease, confirming the earlier observations of McFadden et al. (327). Furthermore, Levy-Frebault et al. (299) showed that wood pigeon mycobacteria could be distinguished from *M. paratuberculosis* and that *M. avium* isolates were readily distinguished from *M. intracellulare*. Coffin et al. (93) used *Ssp*I and pulsed-field gel electrophoresis to identify five RFLP groups in a study of 13 MAC strains. Their results showed that RFLP analysis allows one to readily distinguish between strains of *M. paratuberculosis* isolated from cattle. An *M. paratuberculosis* strain grouped with a serovar 8 MAC strain (the authors apparently mistakenly identified this serovar as *M. intracellulare*), which is consistent with the aforementioned conclusion that *M. paratuberculosis* is most likely a subspecies of *M. avium*. In a large recent study, Mazurek et al. (320, 321) analyzed 72 MAC isolates from 44 patients, including 16 patients with two to five isolates. RFLP patterns generated with *Dra*I were unique for different patients, while multiple isolates from individual patients, including isolates from a variety of body sites, were identical; patterns of multiple isolates were identical over as long as 6 months between isolations (Fig. 1). Arbeit et al. (9) recently reported a similar study of 69 MAC isolates from 14 patients, using the restriction enzyme *Ase*I, but discovered 2 patients who were infected with more than one strain of MAC, suggesting that mixed infections may be common in certain patients or patient populations.

Colony Variant Types

Perhaps one of the most important, yet incompletely understood, features of the MAC is the occurrence of colony type variations. Three colony variants have been described: (i) a smooth, opaque, and domed type; (ii) a smooth, transparent, and flat type; and (iii) a rough type. Clinical isolates of the MAC usually appear as smooth transparent or smooth opaque types or as a mixture of the two. In our experience, MAC isolates from AIDS patients with disseminated disease are frequently exclusively of the smooth transparent type. Barrow and Brennan (16) showed that the rough colony type can be selected by promoting the growth of a pellicle in a broth medium, but once isolated, rough colony types are stable even when repeatedly subcultured on 7H11 agar. They showed that rough colony types lacked both polar and apolar GPLs, and when examined by electron microscopy, rough colony types lacked the sheath (capsule) of fibrillar filaments seen with smooth opaque colony-type cells. Although rough colony variants may occur naturally as an inapparent subpopulation of smooth-type cells, rough forms do not appear to be found in primary isolations from

clinical specimens, and their clinical significance is unknown.

In contrast, the translucent colony variants are reported to be more resistant to antimicrobial agents (391, 411, 486), and there is evidence based on both macrophage and animal studies that this variant is more virulent (116, 335, 407, 420). Stormer and Falkinham (442) isolated nonpigmented colony variants from both environmental sources and clinical material from AIDS patients and showed that these variants were significantly more resistant to antimicrobial agents than pigmented segregants of the same strains. Furthermore, pigmented segregants grew faster on agar media, leading to a concern that the less obvious nonpigmented variants could be overlooked when colonies were being selected for susceptibility testing. Thorel and David (449) showed that there are significant differences in the expression of cell surface antigens between transparent and opaque colony variants; however, such specific differences have not been related to functional differences such as antimicrobial resistance or pathogenicity.

Despite the apparent relationship between colony type and antimicrobial resistance, very little is known about the genetics and regulation of colony type variation. Woodley and David (486) showed that the rate of the transparent-to-opaque transition was dependent on temperature and thus is not a consequence of mutation. The same investigators also indicated that colony type transition was not linked to mutator effects (MAC is not unusually susceptible to UV-induced mutations) or the presence or absence of extrachromosomal genetic elements (124). The rate of transparent-to-opaque transition was 4.6×10^{-4}, while the rate of opaque-to-transparent transition was about 10^{-6} per bacterium per generation (486).

CELL WALL AND ENVELOPE

Structure

One of the best-studied aspects of mycobacteria is the structure and function of the mycobacterial cell wall and envelope which confers upon these unusual bacteria their distinctive feature of acid fastness. The envelope is composed of a variety of soluble proteins, carbohydrates, and lipids and basically three insoluble macromolecular components: arabinogalactan, peptidoglycan, and mycolic acid (329). Together, the insoluble macromolecules constitute the mycoylarabinogalactanpeptidoglycan core of the cell wall, one of two lipopolysaccharides (LPS) common to all mycobacteria. The mycoylarabinogalactanpeptidoglycan appears as electron-dense and electron-transparent zones in thin sections of mycobacteria viewed by negative staining. However, the core is frequently surrounded by additional electron-dense layers at the surface of the cell. This electron-dense layer is made up, in part, of unique GPLs that are specific for the MAC. In addition, all mycobacteria possess a second LPS as a component of the cell envelope, more specifically, a lipoarabinomannan. The lipoarabinomannan is not covalently linked to the mycoylarabinogalactanpeptidoglycan core but most likely is anchored in the plasma membrane of the mycobacterial cell, with the polysaccharide extending to the exterior of the cell. The mycoylarabinogalactanpeptidoglycan, lipoarabinomannan, and GPLs of the MAC are strongly immunogenic, with properties similar to those of the LPS of other bacteria. In addition, certain components of these complex macromolecules have proven to have diagnostic utility; e.g., tuberculostearic acid (10-methyloctadecanoate), which is a useful diagnostic marker for *M. tuberculosis*, occurs as a fatty acid in the lipoarabinomannan of this species. A cartoon of the cell wall structure of mycobacteria that displays the orientations and relationships between the various components of this complex structure is shown in Fig. 2.

McNeil and Brennan (329) discussed the possible relationships between the cell envelope structural features and the noted resistance of the MAC to antimicrobial agents. Clearly, the complex array of parallel hydrocarbon chains is the most likely source of the impermeability of mycobacteria. Camphausen et al. (75) also suggested that these unusual structures were consistent with the long-held conclusion of Rastogi et al. (391, 393) that the antimicrobial resistance of the MAC can be attributed to a lack of drug penetration. Although intrinsic drug resistance is likely to reflect the complex cell wall structure, at the same time these unique structures, amide-linked fatty acids, D-amino acids, and methylated 6-deoxyhexoses, and the corresponding biosynthetic enzymes are excellent potential targets for highly selective and nontoxic antimycobacterial agents.

The impermeability of the MAC cell wall and membrane has been the focus of attempts to potentiate the effect of antimicrobial agents by combining agents with a cell wall-active agent such as ethambutol or a detergent such as Tween 80. Rastogi et al. (392) showed that both ethambutol and an inhibitor of C-mycoside biosynthesis (*m*-fluorophenylalanine) enhanced the activity of other drugs, and Yamori and Tsukamura (496) demonstrated that the activities of rifampin and streptomycin increased in the presence of Tween 80; paradoxically, Tween 80 diminished the activities of ethambutol and sulfadimethoxine.

As mentioned previously, the MAC is a collection of serovars that are distinguished from one another on the basis of antigenic differences in the GPL oligosaccharides. The MAC GPLs, referred to previously as C-mycosides or Shaefer antigens, are alkali-stable molecules, a feature that has been exploited in their analysis, since alkali treatment reduces nonspecific serologic reactions and permits the analysis of whole lipid preparations by ELISAs. In addition, the antigens of other atypical mycobacteria such as *M. kansasii*, *M. xenopi*, and *M. szulgai* are lipo-oligosaccharides that are readily destroyed by alkali (498). *M. simiae* and *M. fortuitum* complex also have alkali-stable GPLs, but there is only limited cross-reaction between these GPLs and those of the MAC. In general, there is good agreement among seroagglutination, thin-layer chromatography, and ELISA; however, some strains remain intractable to analysis by any of these methods, including the monoclonal antibody-based assays. In addition, cross-reactions in the ELISAs are not uncommon and thin-layer chromatography patterns can be indistinct. The type-specific antigens for many of the *M. avium* serovars have been fully described; for example, the structures for serovars 2 and 4 are 2,3-di-*O*-methyl-fucopyranosyl-(a1→3)-L-rhamnopyranosyl-(a1→2)-6-deoxytalose and 4-*O*-methyl-L-rhamnopyranosyl-(a1→4)-2,3-di-*O*-methyl-fucopyranosyl-6-deoxytalose, respectively (66).

Synthesis

Although McNeil and Brennan (329) have proposed a hypothetical biosynthetic pathway for the assembly of the

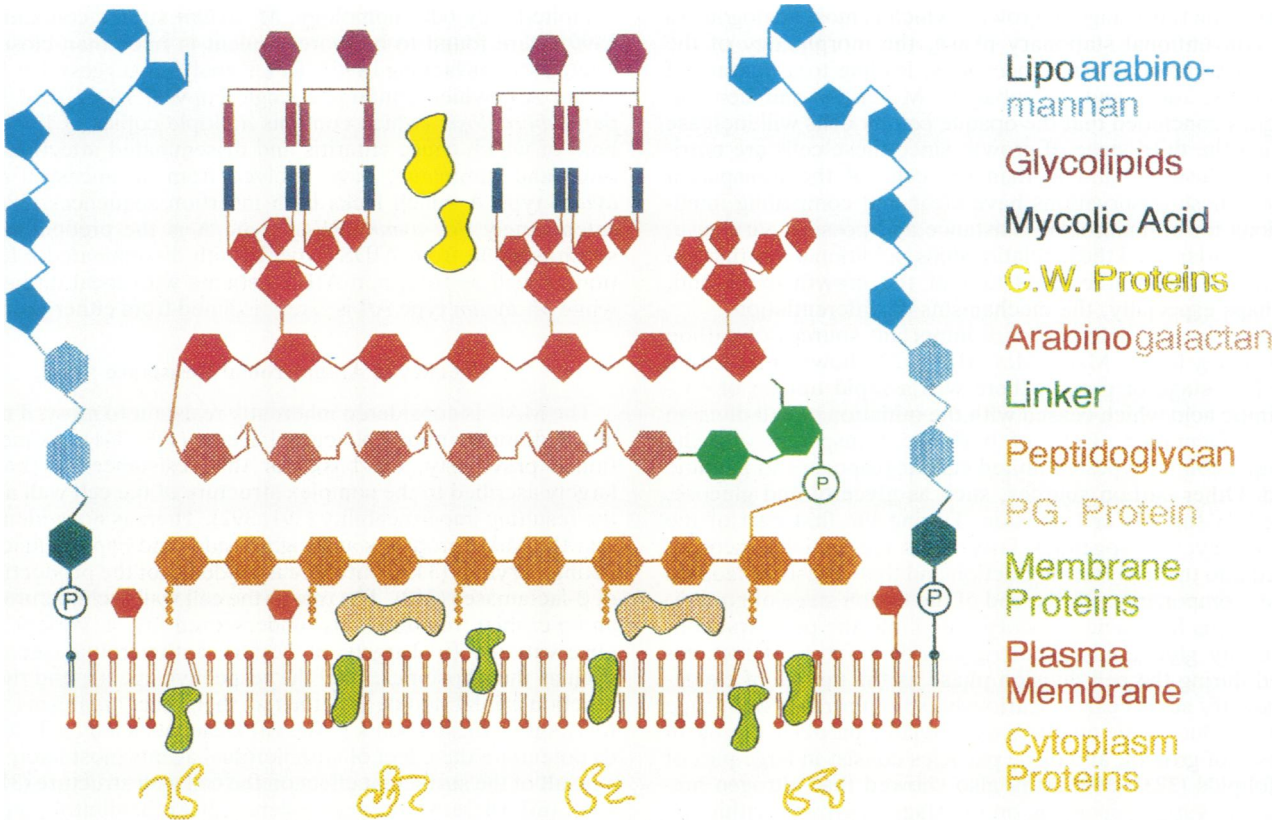

FIG. 2. Schematic representation of the mycobacterial cell wall, taken from McNeil and Brennan (329). MAC serovars are distinguished on the basis of differences in the oligosaccharide component of the glycolipid component. Tuberculostearic acid is a component of the lipoarabinomannan of *M. tuberculosis*. Reprinted with permission of the publisher.

arabinogalactan, including attachment to the peptidoglycan and mycolylation, there is no direct evidence for the biosynthetic enzymes and only a few of the intermediates have been isolated and identified from mycobacteria. Therefore, the recent publication of Belisle et al. (18) on the cloning of genes responsible for the synthesis of GPL antigens must be viewed as a landmark study in the efforts to understand the synthesis of the mycobacterial cell wall and envelope. By using a genomic library prepared from a serovar 2 strain of MAC, the gene cluster responsible for the synthesis of the serovar 2-specific GPL was cloned into *M. smegmatis* mc^2155 with the pYUB18 shuttle cosmid (256). Clones were screened for expression of the serovar 2-specific GPL (antigen) by using a monoclonal antibody directed against this GPL (399). A cluster of genes, designated *ser2*, within a 22- to 27-kb continuous segment of genomic DNA was identified as responsible for the expression of the specific oligosaccharide; chemical analysis revealed that only the oligosaccharide segment arose from the cloned genes. Belisle et al. (18) pointed out that the cloned fragment was larger than necessary to encode the two or three transferases needed to synthesize the oligoglycosyl unit; thus, the fragment may contain multiple operons or other genes. Recently, the same group (130) identified the isomerase that converts ribulose-5-phosphate to arabinose-5-phosphate, which is incorporated into arabinofuranose; this may lead to a better understanding of the mechanism of action of ethambutol since ethambutol is known to disrupt the incorporation of arabinose-5-phosphate into cell wall arabinan.

MICROBIAL PHYSIOLOGY AND GENETICS

Physiology

Considerable basic information about the metabolism and physiology of the MAC is lacking; e.g., there is little or no information about anabolic or catabolic enzymatic pathways, energy metabolism, or carbon and nitrogen cycles. Furthermore, there have been no studies directed at understanding the regulation of macromolecular synthesis or gene expression in this increasingly important group of mycobacteria. As discussed earlier, although there is considerable information about the chemistry of important cell wall constituents, especially antigenic components, there is limited or no information about the biosynthetic pathways. Fundamental work on the growth and nutrition of the MAC, largely from Charlotte McCarthy's laboratory, revealed that the growth of these microorganisms is complex. By studying partially synchronized cultures, McCarthy and Ashbaugh (326) were able to show that the growth of MAC isolates, either transparent or opaque colony variants, occurs in three stages. During the first stage, cells elongate and there is a rapid uptake of fatty acids and an increase in protein and DNA, but without cell division. Binary fission occurs during the second stage of growth, with a generation time as short as 6 h. Protein synthesis continues during the second stage of growth, but at a diminished rate, and the uptake of fatty acids decreases and intracellular pools of triglycerides are catabolized to supply carbon and energy. At the end of the second stage, most cells are in the form of coccobacilli.

During the third stage of growth, which is most analogous to the conventional stationary phase, the morphology of the cells becomes quite heterogeneous, leading to a mixture of filaments, rods, and coccobacilli. McCarthy and her colleagues concluded that the opaque colony cells will increase during the third stage of growth since these cells are nutritionally less demanding than the cells of the transparent type. These observations have clear and compelling implications for antimicrobial resistance and possibly virulence, but to understand these relationships, additional information is needed about the regulation of the growth cycle and, perhaps especially, the mechanisms of differentiation.

Palmitic and oleic acids are important sources of carbon and energy for the MAC. McCarthy (322) showed that during the first stage of growth there was a rapid uptake of ^{14}C-palmitic acid which ceased with the initiation of cell division or fragmentation. Cells of both the transparent and the opaque colony types exhibited similar responses to palmitic acid. Other carbon sources, such as glycerol and glucose, failed to support cell division. During the first part of the growth cycle, exogenous fatty acids are initially incorporated into the triglyceride fraction and then redistributed into other components. By the end of the fission stage of growth, exogenous fatty acid is incorporated into the polar fraction, primarily glycolipids. The triglyceride fraction is metabolized during the cell division phase as the uptake of exogenous fatty acids ceases. Curiously, smooth transparent-type cells produce large numbers of nonviable particles during all phases of growth, and these particles consist in large part of sulfolipids (323). McCarthy also showed that nitrogen metabolism varies depending on the stage of growth within the cell cycle. During elongation, cells were unable to use organic forms of nitrogen such as glutamic acid or glutamine, but they used these amino acids as well as sulfur during periods of rapid cell fission (324). More recently, McCarthy (325) showed that the MAC, including several clinical isolates, preferentially uses ammonia and nitrite and, with the exception of glutamine, does not use amino acids as a source of nitrogen.

Genetics

In those mycobacteria that have been studied in detail, the genome has been found to consist of a single length of DNA in the form of a closed loop. The genome is not contained by a nuclear membrane, although the tightly packed DNA is recognizable on electron microscopy as a nuclear body. Genome size determinations revealed that mycobacteria, compared with most other prokaryotes, have large genomes, in the range of 2.8×10^9 to 4.5×10^9 bp (91). The DNAs of most mycobacteria have between 64 and 70 mol% guanine plus cytosine, and DNA from the *M. tuberculosis* complex exhibited 4 to 25% homology with DNA from members of the fast-growing mycobacterial groups. Extrachromosomal DNA in the form of self-replicating plasmids is common in the MAC (106, 108, 109), but attempts to clearly define the biologic significance of plasmids in *M. avium* strains have been unsuccessful so far. A recent study described an insertion sequence (IS*901*) found in pathogenic strains of *M. avium* but absent in *M. avium* isolates from patients with AIDS (291). The IS*901* insertion element has a nucleotide sequence of 1,422 bp with one open reading frame (ORF1), which encodes a protein of 401 amino acids. It was also determined that the terminal ends and target sites of IS*901* were similar to those of the IS*900* insertion element of *M. paratuberculosis*, while the DNA sequence of both elements exhibited only 60% homology. *M. avium* strains containing IS*901* were found to be more virulent in mice than closely related strains lacking IS*901*. RFLP analyses suggest that *M. avium* A/I (which contains a single copy of IS*901*) and *M. paratuberculosis* (which contains multiple copies of IS*900*), both of which cause enteritis and disseminated infection in birds and ruminants, have evolved from an ancestral *M. avium* type A which lacks both insertion sequences (328). Interestingly, *M. avium* RFLP type A is the predominant strain isolated from AIDS patients with disseminated infection as well as from non-AIDS patients with focal disease, while *M. avium* type A/I is rarely isolated from either group.

Genetics of Antimicrobial Resistance

The MAC is considered inherently resistant to most, if not all, traditional antimycobacterial agents (179, 341). As mentioned previously, the basis for this resistance has been largely ascribed to the complex structure of the cell wall and the resulting impermeability (391, 392). There is no evidence that the MAC produces aminoglycoside- and peptide-inactivating enzymes (341), but there is evidence of the production of β-lactamases (340). The role of the cell wall architecture in antimicrobial resistance is underscored by a variety of observations. (i) Targets of certain antimicrobial agents, such as the ribosomes, bind the respective agents, and their function is inhibited despite the fact that intact organisms are resistant to these agents (341). (ii) Reagents such as Tween 80 potentiate the effect of antimicrobial agents most likely as a result of the surfactant effect on the cell wall structure (316, 496). (iii) There is growing evidence that ethambutol potentiates the activity of other agents (220, 394, 494), and this influence is a consequence of an ethambutol effect on cell wall permeability as evidenced, for example, by microcalorimetric measurements (221).

The colony type has a strong relationship with antimicrobial susceptibility (329, 341, 391), and because colony type transition is not a mutational event, the conversion of antimicrobial resistance phenotypes that is linked to the colony type transition occurs at a relatively high frequency, i.e., 10^{-4} (transparent-resistant to opaque-susceptible) to 10^{-6} (opaque-susceptible to transparent-resistant). Superimposed on this phenomenon is the mutation rate for resistance to specific drugs or heavy metals, which is in the range of 10^{-5} to 10^{-9} per bacterium per generation. The resistance associated with colony type may be considered a type of phenotypic or adaptive resistance and, as such, may be expressed to varying degrees. However, it has been difficult to assess the influence of colony type transitions on some of these measurements. In general, the nontuberculous mycobacteria and MAC, in particular, should be considered heterogeneous, with subpopulations of resistant microorganisms which may range in frequency from 10^{-4} to 1 (125).

EPIDEMIOLOGY

General

Human disease caused by the MAC reportedly occurs worldwide but is predominantly endemic in certain Northern temperate geographic areas, including the United States (180), Canada (170, 250), Great Britain (240), Europe (127, 331), The Netherlands (144), and Japan (338); disease also occurs in Australia (126) and South Africa (356).

Infections with NTM are not reportable in the United States; as a result, the true prevalence of NTM disease is not

known. While *M. gordonae* is the most frequent NTM species isolated from human specimens, MAC is most frequently associated with human disease (38.2 to 73.3% of all pathogenic isolates) (127, 180, 250). The incidence of laboratory isolation of MAC in the United States, based on a 1979 survey of 44 state public health laboratories, is estimated to be 3.2 cases per 100,000 population and was greatest for Hawaii (10.8 cases), Connecticut (8.9 cases), Florida (8.4 cases), Kansas (6.8 cases), North Carolina, Maryland, Rhode Island, and Arizona (180). Several authors have noted an apparent increase in the incidence of NTM infections in the United States and Europe, even when cases in patients with AIDS are excluded (7, 21, 103, 127, 136, 365). In at least two locations, however, the incidence of MAC in non-AIDS patients remained stable or decreased. The rate of isolation of MAC from respiratory specimens at the San Francisco General Hospital steadily increased from 1977 to 1989, but the rate of increase paralleled the increasing incidence of AIDS cases in that city, while the prevalence of MAC isolated in respiratory specimens from non-AIDS patients remained stable (approximately 0.3%) (354). Clinical and laboratory diagnoses of NTM infections actually declined in British Columbia from 1972 to 1981 (250).

Serovar analyses indicate that there are differences in the patterns of human disease-related strains between geographic areas. In the United States, 40 to 50% of the clinical MAC infections in non-AIDS patients are caused by *M. intracellulare*, whereas in western Germany, 81% of the human infections are due to *M. avium* and only 19% are due to *M. intracellulare* (331). In addition, serovar analyses suggest a shift in the proportion of human disease caused by *M. avium* relative to that caused by *M. intracellulare* in certain geographic areas. Meissner and Anz noted that while disease due to intermediate *M. avium* serovars (4 to 6 and 8 to 11) increased from 26 to 71%, the frequency of disease due to *M. intracellulare* decreased from 22 to 5% from 1965 to 1975 in western Germany (331). In Japan, of 661 isolates that caused pulmonary disease, biotype studies indicate a similar significant shift from *M. intracellulare* to *M. avium* in the period 1976 to 1986 (338).

MAC organisms are ubiquitous in nature and can be isolated from natural sources of water, pools, soil, plants and bedding material, and even house dust (158, 243, 396, 467). Surveys of skin test reactivity to antigens prepared from *M. intracellulare* (PPD-B) indicate that the frequency of exposure to this organism is high, particularly in the coastal regions of the southeastern United States and the Gulf, especially in rural areas (>70% in some counties) (140). Data suggest that environmental sources of water constitute the greatest risk of exposure for humans (77, 85, 137, 148, 184, 331, 376, 435, 477), but there are significant gaps in our understanding of the mode of acquisition and pathogenesis of this disease. Indeed, NTM have been isolated from the water supplies of some of the largest metropolitan areas in the United States, including the water supplies of hospitals (85, 138). Drinking water contaminated with MAC was found in 32 of 141 rainwater tanks in Queensland, Australia, but there was no relationship to human disease (458).

Organisms of the MAC may be isolated from both fresh- and saltwater sources (17 to 61% of samples), but recovery is more frequent from waters of moderate salinity (≤2 g% NaCl) and from the Southeast (33%) compared with the Northeast (20%) (148, 190). Studies of soil samples taken from the flood plains of four major eastern rivers demonstrated higher rates of recovery from soil and water samples of relatively high acidity (pH 4.6 to 6.8) and at lower altitudes (69). MAC, but not *M. scrofulaceum*, is found in aerosols in droplet sizes of 0.7 to 3.3 mm above fresh water which is sufficiently small to reach the alveolar spaces after inhalation (477). These studies led the authors to estimate that as many as 18 organisms may be inspired by a human during a 1-h period of exposure. Although isolation from seawater is slightly less frequent than that from fresh water, *M. intracellulare* is highly concentrated within jet droplets released from the air-seawater interface (190). These findings may explain the greater frequency of isolation of *M. intracellulare* from respiratory specimens in some geographic areas.

Plasmids are more commonly found in isolates from surface layer aerosols (75%) compared with isolates identified in soil (5%), dust (7%), and water samples (25%), and the plasmid DNA profiles of aerosolized isolates closely resemble those most commonly isolated from humans (332). A comparison of clinical and environmental MAC isolates revealed that clinical isolates were better able to grow at 43°C without oleic acid-albumin-dextrose-catalase enrichment and more frequently expressed resistance to cadmium compared with environmental isolates, features that closely correlated with the presence of plasmids (158). Only environmental isolates identified in droplets above bodies of water shared those unique characteristics.

M. avium is an important cause of disease in poultry and swine and is commonly excreted in the feces of birds (but not cattle or swine), after which the bacilli can persist in the soil for long periods of time. Although the direct transmission of *M. avium* from animals to humans is thought to be exceedingly rare, epidemiologic analyses of infecting strains suggests that the avian-associated serovars 1 to 3 cause infections in areas where humans and fowl are in close proximity; swine and cattle are even less frequently implicated as the source of infection for humans (331). Additional studies indicate disparity between the serovars that commonly infect humans, poultry, and swine (92, 356). The results of skin test surveys of relatives and housemates of infected persons do not support human-to-human transmission as a significant risk factor (140).

Vaccination with *M. bovis* BCG results in some cross-protection from *M. avium* infection in animals and, possibly, humans. The rate of recovery of viable organisms is lower in BCG-vaccinated mice than in nonvaccinated mice aerogenically challenged with *M. avium* or *M. kansasii*, but not *M. intracellulare* (99, 371). This moderate degree of protection may explain an increase in NTM infections in children following the cessation of community-wide BCG vaccination programs (403).

Patients with AIDS

Since the advent of the AIDS epidemic, immune deficiency due to human immunodeficiency virus (HIV) infection has become the single most significant risk factor for MAC disease. On the basis of AIDS case reporting to the Centers for Disease Control through 1987, the incidence of disseminated MAC as the AIDS-defining illness was 5.5% (233). By December 1990, there were more than 12,000 cases of disseminated NTM infection among the 161,073 patients with AIDS reported to the Centers for Disease Control; of these, the vast majority (96 to 98%) were due to MAC (191, 225, 233). Progressive immunodeficiency due to HIV infection appears to be the single most significant risk factor for disseminated MAC disease (81, 199, 234, 358). The incidences of disseminated disease 1 and 2 years after a diagno-

sis of AIDS, as defined by at least one blood culture positive for MAC, were 21 and 43%, respectively (358). The incidence of disseminated MAC at 1 year was 39% for patients with CD4 counts of <10 per mm^3, but it was only 3% for patients with CD4 counts of 100 to 200 per mm^3 (234). These data correspond to histopathologic evidence of MAC infection in 47 to 50% of patients at autopsy (10, 466). At any given level of immunity, however, the incidence of MAC disease is greater for patients with AIDS compared with those HIV-infected patients without AIDS and is linear over time, suggesting that disseminated MAC may be an inevitable outcome in all HIV-infected patients who do not die of other causes (83, 358).

While there is no apparent age discrimination, disseminated MAC infection is more frequent in Caucasians compared with Hispanic, Haitian, and African-Americans (58, 226, 233, 342, 385). In addition, in patients with HIV infection, disseminated NTM disease is somewhat more frequent in men than in women (9.4 versus 7.0%), in homosexual and bisexual men compared with persons in other HIV risk categories (9.5 versus 6.2%), and in adults compared with children (8.3 versus 5.7%) (226). In contrast, infection due to *M. tuberculosis* is more common in Hispanic, Haitian, and African-Americans compared with Caucasians (58, 226). Inner-city intravenous drug users and women are also more likely to be infected with tuberculosis compared with their homosexual, white male counterparts (151). For example, in a sample of HIV-infected patients with mycobacterial disease, 27 of 45 (60%) Haitian patients were infected with *M. tuberculosis* compared with only 1 of 37 non-Haitians (385).

Despite its emergence as an increasingly common infection in the United States and other developed countries (233), disseminated MAC infection is uncommon in AIDS patients in countries of Africa and other developing nations (58, 345). Serial blood cultures failed to reveal a significant incidence of disease in Ugandan patients with AIDS, even though *M. avium* can frequently be recovered from soil and water specimens in that country (345). This finding may be, in part, due to the significantly higher incidence of tuberculosis and toxoplasmosis in patients with AIDS from developing countries compared with those from developed countries (58). Whereas tuberculosis can occur at any level of immunity, disseminated MAC infection predominantly occurs in patients with profoundly compromised immunity (<50 CD4 cells per mm^3). In geographic areas with inadequate health care and a high incidence of tuberculosis and tuberculosis-associated mortality, patients may not survive long enough to develop disseminated MAC infection.

Environmental strain-related differences also may account for different prevalence rates in various geographic areas. MAC strains isolated from patients with AIDS in the United States and Australia are predominantly serovars 1, 4, and 8 (108, 126, 282, 495). In Sweden, however, while the incidence of disseminated MAC disease is relatively low in patients with AIDS, serovar 6 is most commonly isolated from clinical specimens in that country (219). In Africa, the predominant human and environmental isolate, RFLP type H, does not correlate with any known strain isolated from Western or European AIDS patients (328).

Also, environmental exposure may differ between populations; whereas 98% of MAC infections in AIDS patients are due to *M. avium*, approximately 40% of MAC isolates recovered from patients without AIDS are *M. intracellulare* (191). In addition, *M. intracellulare* made up 13% of respiratory isolates in one large survey of patients with AIDS but only 1.3% of blood isolates and none of the stool isolates (495). Based on RFLP analyses, 73% of the MAC strains recovered from individual patients with AIDS were found to be genetically indistinguishable (194). These intriguing observations suggest that the source of environmental exposure, the route of infection, and other complex host factors, independent of the nature of the infecting strain, may differ in patients with and without HIV infection.

In addition, certain strains of MAC may possess virulence factors that more readily lead to infection and dissemination in patients with HIV infection. In one small study, all 26 strains isolated from persons with AIDS carried plasmids (11 carried one small plasmid and 15 carried two), suggesting that plasmids may play a pathogenetic role in patients with AIDS (108). No data to confirm a role for plasmids in the pathogenicity of MAC are available. The predominant strains isolated from patients with AIDS are, however, serovars 4 and 8, which frequently contain small plasmids or portions of plasmids (215, 265, 344). These plasmids are similar to those identified in serovars 4 and 8 isolated from environmental specimens (265), suggesting that these plasmids may confer specific virulence.

Although MAC organisms can occasionally be isolated from the stools of healthy humans, most are not associated with disease. Furthermore, strains that are more frequently isolated from AIDS patients with disseminated disease are not commonly found in the stools of healthy individuals (194). This observation led investigators to suggest that MAC isolates that cause disease in AIDS patients are not simply gratuitous opportunists but possess specific genetic determinants that confer an ability to penetrate and multiply within macrophages and host cells and contribute to the existing immunodeficiency (194). Implicit in this postulate is the assumption that there are host immune defects, possibly unrelated to the underlying HIV infection, which predispose patients to disseminated infection (39, 54, 114, 115, 224, 352, 446, 457).

PATHOGENESIS

Pathogenesis and the Host

Despite the fact that disease caused by mycobacteria has been known since the time of Koch and that satisfactory therapy exists for most mycobacterial infections, very little is known about the mechanisms of pathogenesis of the most common mycobacteria that cause disease, including *M. tuberculosis*, *M. leprae*, the MAC, and *M. kansasii*.

While humans are highly susceptible to *M. tuberculosis* and *M. leprae* infection, most people who are exposed to these bacteria never develop clinical disease, indicating that the normal immune system can control these microorganisms (86, 483). This observation is even more applicable to exposure to MAC organisms because, despite evidence of exposure rates as high as 70%, the incidence of clinical disease is remarkably low (<10 cases per 100,000 population). Before the AIDS epidemic, pulmonary infection was the principal, albeit infrequent, manifestation of disease. Dissemination of infection was unusual and, with rare exception, occurred in persons with defects in cellular immunity. However, even in severely immunocompromised individuals, such as those with hairy cell leukemia who seem to be predisposed to MAC infection (21, 64, 318, 397, 440, 476, 481), the incidence of MAC infection is only 5%. In contrast, *M. avium* appears to have a particular predilection for infecting and disseminating within HIV-infected patients.

Routes of Infection

The most likely route of penetration of opportunistic mycobacteria into tissue is across the bronchial or intestinal mucosa. Current evidence points to the intestinal tract as the primary route of *M. avium* infection in AIDS patients (186, 287, 342, 436, 466) and the respiratory tract as a secondary and significantly less frequent portal of entry (4, 231, 257, 388). Disseminated disease in AIDS patients is frequently preceded by gastrointestinal tract colonization (22, 87, 231, 388) as evidenced by the relatively high frequency of positive stool cultures (22, 120, 202, 231, 342, 436) and the high frequency of gastrointestinal involvement, with large numbers of mycobacteria infiltrating the intestinal mucosa and submucosa (120, 186, 287, 406). A study of AIDS patients by Damsker and Bottone (120) was one of the first to suggest that colonization of the intestinal tract preceded the development of bacteremia. Other work supports this concept and, indeed, colonization of the intestinal tract with *M. avium* in patients with AIDS was shown to precede the appearance of bacteremia and disseminated disease by 4 to 5 months (53). Massive Peyer's patch and mesenteric lymph node involvement is a common histopathologic finding in these patients, along with intestinal erosion and chronic diarrhea (120, 287, 405, 406, 484).

Although there is little direct evidence that *M. avium* disseminates from the lung, one study showed that sputum cultures were twice as likely to be positive as stool cultures (66 versus 33%) (231). Progression to dissemination occurred with equal frequency (33%) in patients with positive respiratory or stool isolates during a mean observation period of 5 months. Recent data presented by Chin et al. (87) indicated that nearly 75% of patients develop mycobacteremia within 1 year (median duration time of 6 months) after the isolation of MAC organisms from respiratory secretions or stool. Nevertheless, of those patients who developed MAC bacteremia, only 25 and 36% had a preceding positive respiratory or stool culture, respectively. These data suggest that the methods available to screen for gastrointestinal tract colonization lack sufficient sensitivity, resulting in a poor negative predictive value.

Asymptomatic respiratory and intestinal colonization with *M. avium* can be seen in healthy individuals, but the development of focal or disseminated disease in them is rare. Ingestion of mycobacteria in water or food appears to lead to colonization of the intestinal tract (100, 309). Our studies with a beige (C57BL/6 *bg+/bg+*) mouse model of oral infection demonstrated that oral exposure of *M. avium* strains isolated from AIDS patients leads to intestinal colonization and subsequent dissemination of infection. Detailed studies of bacterial localization along the gastrointestinal tract showed that the great majority of the organisms are found in the terminal ileum and ascending colon (34). Concomitant ingestion of a mucosal irritant, such as ethanol, led to an increased colonization of the upper gastrointestinal tract, with a large number of bacteria being cultured from the stomach and mucosa and submucosa of the proximal intestines. Once in the intestinal lumen, the bacteria bind to enterocytes and probably M cells and quickly penetrate the intestinal epithelial cells before translocating into the lamina propria. The bacteria can colonize Peyer's patches and are eventually found localized in the liver and spleen as well as circulating in the blood.

It is possible that factors such as gastric achlorhydria and the use of oral antibiotics facilitate the colonization by *M. avium*. Studies in animals support this hypothesis, although even closely related mycobacterial species can exhibit wide variations in mouse virulence when introduced by the oral route (34).

Invasion of Mucosal Cells

We showed that AIDS-related *M. avium* strains can bind and invade HT-29 cells, a well-differentiated human intestinal epithelial cell line, in a manner that is likely to mimic the attachment and invasion of mycobacteria to the gastrointestinal tract of humans (41). Non-AIDS-related *M. avium* strains also bind and invade but are less efficient than AIDS-related strains. In addition, *M. avium* can bind and invade both human oropharyngeal cells and the HEp-2 oropharyngeal cell line (41). In more recent studies, we injected mycobacteria into the intestinal lumen of an isolated segment of the terminal ileum of C57BL *bg+/bg+* mice being kept alive under anesthesia. Following different periods of exposure, we performed quantitative cultures on a 2-in. (ca. 5-cm) segment of the terminal ileum to measure the number of bacilli associated with mucosa and submucosa. In these experiments, we found that *M. avium* rapidly bound to and invaded the intestinal mucosa; however, this feature was strain specific. Strain MAC 101 colonized and invaded more rapidly than another AIDS-related strain (MAC 107, a serovar 8 strain) (236). Histopathological studies, in which hundreds to thousands of mycobacteria were observed in intestinal macrophages, clearly confirm that disease-associated strains of *M. avium* readily invade the intestinal mucosa and submucosa (236). Less virulent serovars of *M. avium* also possess the necessary cell wall adhesion but probably lack accessory virulence factors and do not survive within tissue macrophages.

Recent studies of *M. tuberculosis* demonstrated that the ability of tubercle bacilli to invade HeLa cells is encoded in a 3-kb genomic DNA fragment (11). In a parallel series of experiments, we used an *M. avium* library of chromosomal DNA to transform *Escherichia coli* K-12, which cannot invade cultured mammalian cells. *E. coli* transformants that had acquired a 2.7-kb fragment of chromosomal DNA and the ability to bind and invade human HT-29 and HEp-2 cells were isolated. Thus far, we have evidence for the presence of at least one adhesion protein in virulent strains of *M. avium*. Specific antibody generated with a purified preparation of a 27-kDa putative adhesion protein blocked the binding of *M. avium* strains to both intestinal and oropharyngeal mucosal cells (236). However, it seems clear from our studies and studies of *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Salmonella cholerae-suis* that the ability to penetrate epithelial mucosal cells can proceed by a number of pathways (154, 251). In the case of *Salmonella* sp., Finlay and Falkow (154) showed that a cluster of proteins is synthesized in response to contact with MDCK cells. A mutation that blocks the synthesis of these proteins interferes with the ability of salmonellae to bind and enter mammalian cells; by extrapolation, intestinal cells may play an active role in taking up *M. avium*. Indeed, a 47- to 50-kDa glycoprotein present on HT-29 cells and oropharyngeal cells binds to the putative 27-kDa *M. avium* adhesion protein and appears to be involved in the binding of several different *M. avium* strains. However, it must be emphasized that in AIDS patients a number of factors probably facilitate *M. avium* infection and invasion of the intestinal mucosa, including coinfections with cytomegalovirus and HIV type 1.

Infection of Nonprofessional Phagocytes

Virulent MAC strains can invade and survive within cells other than mononuclear phagocytes and epithelial cells. This ability to infect a variety of cell types may be related to the persistence of infection in the immunocompromised host; e.g., Bermudez (26) demonstrated that MAC organisms can infect and grow within fibroblasts and, once inside the fibroblast, the mycobacteria are protected against nonspecific mechanisms of killing. Once the bacilli are intracellular, major histocompatibility class I-mediated or NK cell-mediated cytotoxicity is necessary to release the bacilli from the cells. Although it is difficult to assess the overall importance of the ability of MAC to infect "nonprofessional" phagocytes, it is plausible that in a setting of profoundly impaired cytotoxic response the ability to invade fibroblasts, endothelial cells, and other nonprofessional phagocytes contributes to persistence and dissemination.

Interaction with Mononuclear Phagocytes

Intracellular pathogens typically reside within a niche of the host where the pathogen can multiply or survive the onslaughts of cellular and humoral defense mechanisms. Thus, the armamentarium of pathogens includes mechanisms that counteract both nonspecific and specific host defenses. Studies with *M. tuberculosis* (421) and *M. leprae* (422) demonstrated the importance of complement receptors (CR1 and CR3) for the binding and phagocytosis of mycobacteria. In addition, it is now clear that several species of mycobacteria including *M. leprae*, *M. tuberculosis*, and *M. bovis* bind to serum fibronectin by way of a 30-kDa receptor (1). Phagocytosis of the MAC by monocytes and macrophages occurs mainly via the CR3 receptors in both the presence and the absence of serum (47, 401). In addition, MAC organisms bind to serum fibronectin and the bacilli are internalized by macrophages by using the integrin fibronectin receptor. The use of an Fc receptor-independent pathway for uptake may offer significant advantages for the invading microorganism. For instance, a study by Wright and Silverstein (488) showed that phagocytosis with Fc receptors, but not complement receptors, is associated with superoxide anion production by phagocytic cells, and invasion by other mechanisms may influence the structure and function of the intracellular vacuole. Although the ability to multiply inside mononuclear phagocytes is not uniform among *M. avium* strains, AIDS-associated strains remain viable within human and murine macrophages (39, 116) and resist the respiratory burst-associated bactericidal mechanisms (42, 166). MAC as well as *M. tuberculosis* synthesize a 23-kDa superoxide dismutase that can inactivate macrophage-derived superoxide anion (319) and other proteins, such as the 65-kDa heat shock protein, which are powerful inhibitors of superoxide anion production (30). The origin of this activity is unknown, but the 65-kDa protein is released in large quantities in response to stress conditions such as high temperature, change in pH, and phagocytosis (30). The survival of pathogenic strains of *M. avium* within macrophages also is related in part to their capacity to inhibit fusion of the phagosome and lysosome, thus preventing contact with proteolytic enzymes (54, 115). In the absence of phagolysosome fusion, the intracellular environment of the macrophage remains neutral or alkaline, which may directly influence pathogen survival and the effectiveness of certain antimicrobial therapies. Studies by Frechel and colleagues (156) suggested a role for cell surface components, other than the C-mycosides, in this phenomenon. Walker and Lowrie (465), using *M. microti*, proposed a role for cyclic AMP and prostaglandin E_2 in phagolysosome fusion.

IMMUNE RESPONSE

Cellular Immunity

Mycobacteria are considered the archetypical intracellular pathogens because of the capacity of these bacilli to invade and multiply within macrophages (39, 116); thus, the cellular immune response to mycobacterial infection has been a subject of considerable study. Phagocytosis and processing of antigens by macrophages or B lymphocytes trigger a specific cellular immune response, including the activation of T-helper cells, macrophages, T-cytotoxic cells, and NK cells. Antigen processing occurs after infection with mycobacteria and leads to a complex host response involving multiple arms of the immune system (427, 428). Although there is evidence that $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and $\gamma\delta$ T cells (197, 237, 348) are important factors in this response, there is surprisingly little information about the cellular immune response to NTM. For example, some strains of the MAC induce a chronic, lifelong lung infection in normal mice (102), while more virulent MAC strains cause a disseminated disease associated with high mortality (163). Most strains of *M. intracellulare* are less virulent and tend to induce chronic pulmonary infections in C57BL/6 or beige mice (370), but infection with these strains is exacerbated by the absence of T cells such as in congenitally athymic *nu/nu* mice (101). In mice, resistance to early growth of *M. bovis*, *M. lepraemurium*, *M. intracellulare*, and *M. avium* may be controlled by a single dominant autosomal gene, *Bcg* (424). Furthermore, phagocytosis- or ligand-induced respiratory burst activity is significantly greater in macrophages from resistant animals than in macrophages from susceptible mice (372), and the transfer of immune cells (T or NK cells) from resistant to susceptible mice is associated with an increased ability of the latter animals to control *M. avium* infection (31). However, these observations contradict the observation that peritoneal macrophages from MAC-resistant and MAC-susceptible mice have equal capacities to ingest and inhibit or kill MAC in vitro (31).

Role of Cytokines

Both cultured mouse and human macrophages can be stimulated by cytokines to inhibit or kill intracellular *M. avium*. Bermudez and coworkers (45, 51) and Denis and Gregg (132) showed that recombinant tumor necrosis factor (TNF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) induce mycobacteriostatic and/or mycobactericidal activity and that macrophages stimulated with TNF or GM-CSF respond differently than unstimulated macrophages. In stimulated macrophages, there is an increased release of superoxide anion and phagolysosome fusion occurs more frequently following uptake of mycobacteria (54). Although the mechanisms of inhibition and killing of intracellular MAC are not completely understood, the nonoxidative mechanisms of defense have an important role (27, 42). Bactericidal proteins purified from human macrophages increase the permeability of the mycobacterial cell, and this effect is ultimately bactericidal (40). Although the nitric oxide-dependent pathway of intracellular killing is associated with the nonoxidative killing of a number of intracellular pathogens such as *Toxoplasma gondii* (3) and *Leishmania*

major (187), there is controversy about the role of this pathway in the killing or inhibition of mycobacteria. Some studies suggest that this pathway is important in the killing of the MAC (131), *M. leprae* (2), and *M. tuberculosis* (84), while other studies show that this pathway is not important for the killing or inhibition of MAC growth (27).

The ability of cytokines to stimulate macrophages and inhibit growth of the MAC depends on the strain; e.g., gamma interferon (IFN-γ) stimulates inhibition or killing of certain non-AIDS strains of MAC (44), but not that of AIDS-related MAC strains (51). Paradoxically, the administration of recombinant TNF or GM-CSF was associated with increased killing of mycobacteria in the beige mouse model of disseminated MAC infection (32, 35), but macrophage monolayers infected with MAC organisms for longer than 48 h failed to respond to these cytokines even when the cytokines were administered 7 days after infection (44). The conclusion was that response to cytokine stimulation reflects the influence of MAC infection on the ability of macrophages to respond to cytokines. In addition, the MAC can interfere with other cytokine pathways by stimulating the production and release of suppressor molecules such as interleukin-6 (IL-6) and transforming growth factor β (TGF-β) or by directly influencing the mechanism of signal transduction within macrophages (28, 38). The release of inhibitory cytokines by MAC-infected macrophages occurs within a few days of infection, and infection with AIDS-related strains of the MAC stimulates the release of IL-6 and a suppression of macrophage function by down-regulating the expression of TNF receptors and decreasing TNF production (35, 57, 132). There is some evidence that IL-6 stimulates growth of the MAC (426); however, IL-6 binds not only to virulent MAC strains, but also to nonvirulent strains, *M. smegmatis*, *E. coli*, and *Pseudomonas aeruginosa*, raising the possibility that the influence of IL-6 binding is nonspecific (35).

M. avium-infected macrophages release TGF-β soon after infection (28), and the 61- and 33-kDa cell surface proteins of MAC stimulate macrophages to release TGF-β in culture (28). Furthermore, much of the TGF-β released from infected macrophages is in the active form, whereas TGF-β released from control macrophages is in the inactive form. Since TGF-β is known to impair the ability of macrophages to respond to cytokines, the release of TGF-β is likely to be responsible for the lack of response to IFN-γ by MAC-infected macrophages. A specific amino acid sequence within the 33-kDa protein interferes with the regulation of transcription in macrophages, which in turn influences the response of macrophages to stimulation with TNF-α (29). Interference with cytokine-related signal transduction and transcription is probably an important mechanism of pathogenesis and contributes to the persistence of MAC within macrophages.

Thus far, the results of various studies directed at understanding the nature of the interaction between HIV type 1 and *M. avium* within macrophages are inconclusive. In some studies of coinfected macrophages, HIV type 1 did not affect the *M. avium* infection, while others claim that *M. avium* grew significantly faster in HIV-infected macrophages (262, 267). Peripheral blood mononuclear phagocytes obtained from AIDS patients are not functionally impaired (334), since these phagocytes respond to stimulation with cytokines several days after being harvested from blood; however, phagocytic cells obtained from the peripheral blood may be significantly less impaired than heavily infected tissue macrophages.

Finally, a number of cofactors could contribute to the impairment of macrophage function in AIDS patients. Alcohol ingestion is common in some at-risk population groups (315). Because of the relationship between alcohol consumption and pulmonary tuberculosis (70, 238), it is plausible that there is an association between alcohol consumption and *M. avium* infection in AIDS patients. Human macrophages exposed to serum-achievable concentrations of ethanol are more permissive to intracellular growth of *M. avium* than macrophages not treated with ethanol (46), and ethanol both impairs the ability of macrophages to respond to stimulation with TNF and GM-CSF (37) and may act as a mycobacterial stress factor (43). Other drugs of abuse impair macrophage function. Peterson et al. (381) demonstrated that treatment of macrophage monolayers with cocaine was associated with increased replication of HIV type 1; however, the relationship between this observation and infection of macrophages with MAC organisms is unknown.

Role of Lymphocytes

Several studies have shown that T lymphocytes are important in the immune response to mycobacterial infections including the generation of a positive skin hypersensitivity response to intradermal administration of PPD. In the infected host, expression of class II molecules by infected macrophages results in the presentation of mycobacterial antigens to class II-restricted $CD4^+$ T lymphocytes of the helper/inducer type. Mice inoculated with a crude lysate of *M. tuberculosis* or *M. avium* will respond with a proliferation of T cells specific for mycobacterial antigens. In a limiting-dilution analysis, approximately 20% of the $CD4^+$ T lymphocytes that were reactive to mycobacterial antigens recognized the mycobacterial 60-kDa heat shock protein (272).

Little is known about the role of T cells in preventing the growth of *M. avium* in the tissues of immunocompetent hosts. While T-cell depletion enhances the severity of infections with some MAC strains, T-cell depletion does not affect the severity of infection with other strains (101). Depletion of the $L3T4^+$ or $Lyt\text{-}2^+$ T-cell subpopulation does not have any significant effect on the immune response to *M. avium* in mice, but depletion of both subsets ablates the immune response (237). However, it appears that the interaction of activated $CD4^+$ T cells with macrophages does not have the same effect with *M. avium* as with *M. tuberculosis* (271).

The antibody response of humans and mice to MAC infection, as judged by Western blots (immunoblots), is heterogeneous (36, 343), and blot profiles from different patients show distinct individual patterns with a few predominant common bands. Like *M. tuberculosis*, *M. avium* releases a 65-kDa protein in response to the stress of increased temperature or exposure to acid pH (39). The release of proteins in response to stress has been demonstrated in nonmycobacterial systems, and Young and Garbe (501) speculated that the 65-kDa protein of *M. tuberculosis* is an analog of the GroEL protein in *E. coli*. Other mycobacterial antigens, including the 71-, 65-, 38-, 33-, 30-, and 10-kDa proteins, can be released and recognized by $CD4^+$ and $CD8^+$ T lymphocytes. In addition, mycobacterial glycolipids can have an immunomodulatory effect (447) and certain GPLs from *M. avium* can interfere with the lymphoproliferative response (72). T cells bearing the γδ T-cell receptor appear to have a special role in the immune defense system and there is a strong correlation between infection with an intracellular pathogen and the accumulation of γδ T cells at

the sites of infection, including the skin and lung epithelium. The relationship between γδ T cells and invading microorganisms is central to the hypothesis of immunosurveillance and suggests that a primitive subset of T cells provides an initial line of immune defense by recognizing highly conserved molecules produced during environmental stress; i.e., γδ T cells with a severely restricted receptor repertoire react to the release of highly conserved mycobacterial 60- and 70-kDa heat shock proteins. O'Brien et al. showed that γδ T cells proliferate in response to PPD and to a recombinant 65-kDa protein of *M. tuberculosis* (368), and our preliminary studies confirmed these findings and extended the observation to *M. avium*; i.e., γδ T cells from four different donors lysed infected target cells in a major histocompatibility complex-independent manner after stimulation with *M. avium* (33).

The role of human γδ T cells in the first line of immune defense to mycobacterial infections also is strongly suggested by the accumulation of γδ T cells at the site of granulomatous responses to *M. leprae* (462) and in tuberculous lymphadenitis (258). Among the γδ T-cell subpopulations there is evidence that the response to pathogenic *M. tuberculosis*, *M. avium-M. intracellulare*, and *M. scrofulaceum* is mainly confined to the Vδ9 Vδ2 TCR-positive cells, and the selective triggering of these T cells may reflect a super-antigen-like effect of mycobacterial antigens or antigen-specific stimulation, possibly in response to the heat shock protein 60 (196, 367).

Role of NK Cells

The role of NK cells in the immune response to *M. tuberculosis* and *M. avium* has been well established in a variety of studies (50, 55, 270). More recent findings indicated that NK cells are cytotoxic in a nonrestricted manner and stimulate mycobacteriostatic and mycobactericidal activities in infected macrophages (50, 55, 270). The cytotoxicity of NK cells for infected macrophages appears to depend on binding via the LFA-1 glycoprotein receptor (56); however, the validity of this observation could be questioned since other recent studies showed that NK cells do not efficiently bind or lyse target cells expressing the class I major histocompatibility complex. It is of interest to note that Blanchard and colleagues (57) showed that NK cells exposed to *M. avium* release large amounts of IL-6, which may have an important influence on the host immune response.

The mechanism by which NK cells stimulate macrophages appears to be mediated by the release of cytokines. Gomez et al. (176) and Pohjdak et al. (387) demonstrated the ability of activated NK cells to produce cytokines that activate macrophages, and studies of the role of NK cells in the inhibition and killing of MAC organisms are in agreement with these previous studies (50). TNF, IFN-γ, and GM-CSF are secreted by activated NK cells and, theoretically, can influence macrophage activity. Anti-TNF antibody, but not anti-GM-CSF antibody, partially blocks the stimulatory effect of the supernatant fraction of activated NK cells but cannot block the effect of purified NK cells. This observation suggests that the effect of NK cells on macrophages occurs through direct cell-to-cell contact (50). Numerical and functional deficiencies of NK cells in patients with AIDS (61) may account for the ability of *M. avium* to invade and establish infection in tissues. This hypothesis is supported by a recent study that showed that C57BL/6 mice treated with antibody to deplete NK cells developed a more severe form of disseminated disease than untreated mice (197).

CLINICAL DISEASE IN PATIENTS WITHOUT AIDS

Pulmonary Disease

The first case of human disease due to *M. avium* was reported in 1943 in a middle-aged underground miner from the Mesabi Iron Range of Minnesota in what became a classic description of pulmonary disease due to this organism (152). During the next two decades, a number of cases of MAC pulmonary disease were reported (113, 300, 451), and until the emergence of AIDS, lung infection alone was the most common presentation of disease due to this organism.

Pulmonary disease due to *M. avium* predominantly involves white males 45 to 65 years of age with preexisting pulmonary disease (144, 145, 404, 468), but there is tremendous variation in the sex, age, and race of these patients. Predisposing conditions such as chronic obstructive pulmonary disease, bronchiectasis, chronic aspiration or recurrent pneumonia, inactive or active tuberculosis, pneumoconiosis, and bronchogenic carcinoma are present in 54 to 77% of patients with pulmonary MAC disease (144, 145). Also, MAC organisms are frequently recovered from adults with cystic fibrosis, particularly in the southeastern United States (283). Differentiation of infection from the coexistent pulmonary disease may be difficult, and the clinical and radiographic presentation may be indistinguishable from tuberculosis (373). A positive tuberculin skin test may be helpful in differentiating the two processes; however, coinfection with *M. tuberculosis* and *M. avium* has been demonstrated (456).

The symptoms are varied and nonspecific, commonly including chronic productive cough, dyspnea, sweats, malaise, fatigue, and, less commonly, hemoptysis. Fever and weight loss are not common but may occur. Approximately 75% of patients have evidence of cavitary infiltrate on chest roentgenograms, typically involving the apical and anterior segments of the upper lobes, but dense unilobular or multilobular infiltrates, diffuse interstitial or reticulonodular infiltrates, or a solitary pulmonary nodule may occur (89, 188, 310, 389). Cavities or infected bullae tend to be thin walled with less surrounding infiltrate than that associated with tuberculosis. Bilateral involvement is common and there may be a dense pleural reaction, but pleural effusions are unusual. The radiograph of a patient without AIDS and one of a patient with AIDS, both with pulmonary disease, are shown in Fig. 3 and Fig. 4 and may be compared.

MAC organisms may be isolated from the sputum in the absence of apparent disease, particularly in patients with chronic respiratory disorders; such low-grade infection or colonization is more common than true disease. These patients may have episodic excretion of organisms which frequently clears with good pulmonary toilet. Also, isolation of the organism may simply represent contamination of the specimen, and care must be exercised in interpreting the results of these cultures.

Guidelines have been suggested for distinguishing patients with NTM lung disease from patients who are simply colonized. NTM disease in patients with noncavitary infiltrates can be assumed to be present when (i) two or more sputum or bronchoalveolar wash specimens are smear positive and/or result in moderate to heavy growth in culture; (ii) sputum cultures fail to revert despite good pulmonary toilet or 2 weeks of antimycobacterial therapy; and (iii) reasonable attempts fail to identify other underlying causes of disease

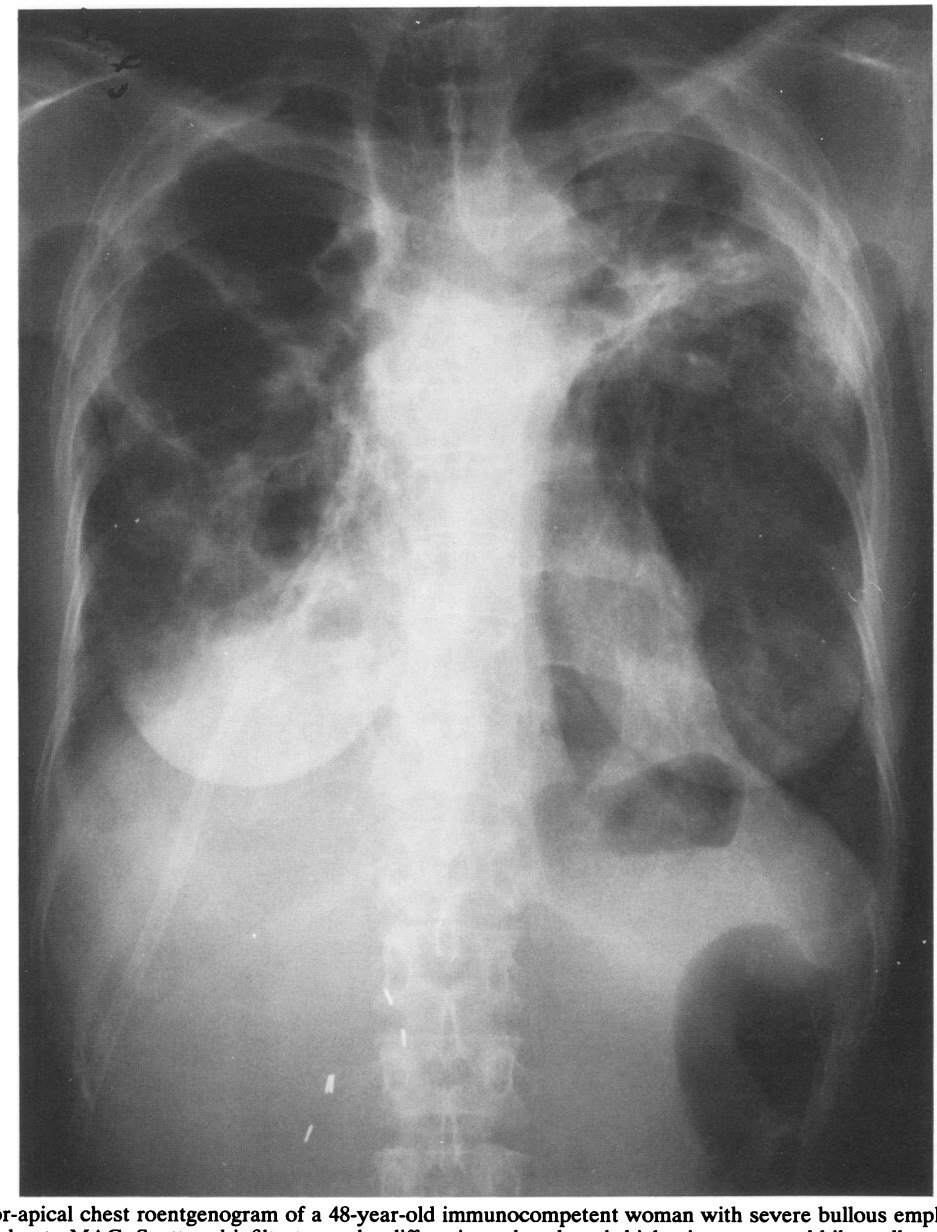

FIG. 3. Posterior-apical chest roentgenogram of a 48-year-old immunocompetent woman with severe bullous emphysema and extensive pulmonary disease due to MAC. Scattered infiltrates and a diffuse irregular pleural thickening are noted bilaterally.

(468). It is important to note that these guidelines may not apply to immunodeficient patients.

More individuals, especially women, in previously good health and without the usual predisposing conditions are being recognized with pulmonary MAC disease (252, 389, 395). Many of these patients were older women with indolent symptoms and atypical radiographic features (e.g., a solitary nodule), which frequently resulted in a delayed diagnosis. Six of 29 patients, all female, with *M. avium* pulmonary infection had isolated middle lobe or lingular involvement (395). The authors termed this the Lady Windermere's Syndrome, after Oscar Wilde's Victorian character, because many patients had a fastidious habit of cough suppression that the authors considered a potential predisposing factor. In another series, 4 of 21 patients died from progressive pulmonary MAC infection, and none of these patients had an underlying immunodeficiency or other contributing disease (389). The prognosis of *M. avium* pulmonary disease may be worse than that of *M. intracellulare* disease. In one survey, 3 of 28 patients with *M. avium* died and 1 was cured, while none of 27 patients with *M. intracellulare* died and 6 were cured of disease (497).

Patients with an underlying immunodeficiency are at risk for pulmonary MAC disease, including those compromised by cytotoxic chemotherapy, corticosteroids, or allogeneic bone marrow, renal, or cardiac transplantation. Such patients commonly present with atypical radiographic features (292), and attempts at establishing a diagnosis may be difficult and complicated by the presence of multiple pathogens. Also, the administration of steroids may mask the clinical symptoms. Pulmonary MAC disease in children is rare and is usually a component of disseminated infection in

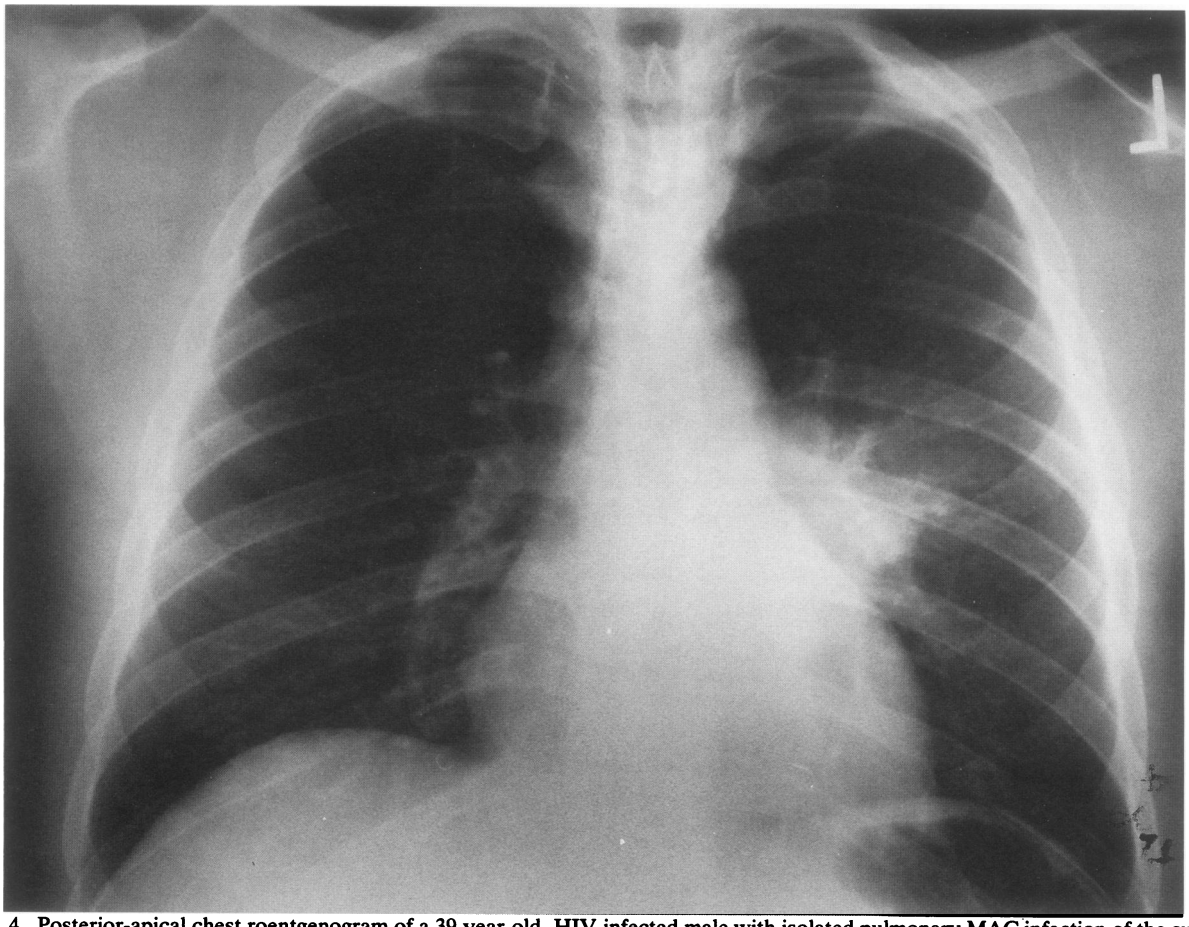

FIG. 4. Posterior-apical chest roentgenogram of a 39-year-old, HIV-infected male with isolated pulmonary MAC infection of the superior posterior portion of the left lower lobe. Note the absence of a pleural effusion or scarring, cavitation, or hilar lymphadenopathy.

the presence of an underlying immunodeficiency (216, 305). The role of MAC in one special pediatric population, cystic fibrosis patients, is unclear; however, the isolation of MAC organisms from respiratory secretions of older cystic fibrosis patients is not uncommon (283).

In most cases, the diagnosis of MAC pulmonary disease can be established without lung biopsy, but in patients with low numbers of organisms or an unexplained or atypical radiographic presentation, percutaneous, transbronchial, or open lung biopsy may be necessary. A recent study identified MAC organisms in the sputum of 11 of 97 patients with active tuberculosis (cultures were first incubated at 42°C for 3 weeks to suppress the growth of *M. tuberculosis*) (456); however, the clinical significance of *M. avium* in these patients was not entirely clear. Antibodies to *M. avium* detected in serum or pleural fluid are not specific, and antibodies in the pleural fluid most likely reflect passive diffusion from sera and not localized production (298).

Histopathologic presentations are varied; both caseating and noncaseating granulomatous necroses are common and may be associated with a granulomatous bronchitis. Ill-formed granulomata with histiocytic reaction are more commonly reported in immunodeficient patients but also are seen in immunocompetent hosts. A granulomatous vasculitis (histologically similar to Wegener's granulomatosis) or a nonspecific interstitial pneumonitis with organizing pneumonia may be the only finding (310). A survey of 20 resected solitary pulmonary nodules with histologic evidence of granulomata and acid-fast bacilli indicated that 12 (60%) were due to MAC, 1 was due to *M. tuberculosis*, and 5 were culture negative (188).

Subacute Lymphadenitis

Granulomatous inflammation accounts for approximately 20% of cases of upper anterior cervical, submandibular, submaxillary, and pre-auricular lymphadenopathy in children 1 to 5 years of age. Most of these cases are the result of infection due to the MAC, *M. scrofulaceum*, or the etiologic agent of cat scratch disease. Mycobacterial lymphadenitis usually presents as an insidious, painless, unilateral process involving one or more nodes in a regional distribution (20, 170, 264, 293, 417, 468); axillary and inguinal nodes are occasionally involved (98). Spontaneous sinus tract formation occurs in approximately 6% (264). Children above the age of 12 years are rarely infected except in circumstances of immunodeficiency or disseminated disease (293).

Of the mycobacteria isolated from the nodes of children which can be characterized, 63 to 80% are MAC, 10 to 20% are *M. scrofulaceum*, and approximately 10% are *M. tuberculosis* (20, 170, 264, 293, 468). These findings are in distinct contrast to mycobacterial lymphadenitis in persons older than 12 years, 95% of which is due to infection with *M.*

tuberculosis and only 3% of which is due to infection with MAC organisms (293).

While more than 90% of children with mycobacterial lymphadenitis have a positive skin test reaction to PPD-B (264), safe, standardized, antigenic material is not available for routine diagnostic purposes. Such patients would, however, have a negative tuberculin skin test, unless they were coinfected with *M. tuberculosis*. Fine-needle aspiration may yield the diagnosis with a positive culture in less than 50% of cases (8, 438). Histopathologic presentations of lymphadenitis are varied but generally demonstrate caseating granulomatous inflammation and necrosis, epithelioid histiocytes, and occasional giant cells. Rarely, histiocytic inflammation and a lack of granulomatous inflammation occur, chiefly in children with selective immunodeficiency (347).

Disseminated Infection

An excellent review of disseminated MAC infection in non-AIDS patients is provided by Horsburgh et al. (230), and several comprehensive reviews of the spectrum of clinical disease have been provided elsewhere (253, 305, 468, 482, 487); the following presents a brief overview of disseminated disease and of unusual sites of involvement.

Disseminated infection with the MAC was extremely unusual prior to the AIDS epidemic. Typically, the disease occurred in individuals with underlying malignancy or inherited or therapeutic immunodeficiency, especially children and young adults with hematogenous malignancy or severe combined immunodeficiency syndrome, transplant recipients, and patients receiving cytotoxic chemotherapy or corticosteroids (230, 361, 468, 482). A specific immune defect may predispose patients with hairy cell leukemia to disseminated disease (64, 318, 397, 440, 476, 481). In one large series, 5% of patients with hairy cell leukemia were diagnosed with NTM infections (21), and other data indicate an association of disseminated NTM infections in cardiac transplant recipients with prior nocardiosis (430). Holland (223) recently described a possible X-linked deficiency of CD45RO cell IFN-γ production in a child with disseminated MAC infection; two maternal uncles of the child had chronic disseminated MAC infection and abnormally low levels of IFN-γ.

The most frequent presentation of disseminated infection in the immunocompromised host is fever of undetermined etiology. Dissemination may involve any organ system but, most commonly, the lungs and large airways, the mononuclear phagocyte system including the liver, spleen, and retroperitoneal nodes, the gastrointestinal tract, the skeletal system, and the skin (76, 157, 230, 305, 415, 482). Rarely, the brain, cerebrospinal fluid, and orbit are involved (147, 282, 305, 459). Mycobacteremia was seldom reported previously, but with the improvement in isolation techniques, bacteremia may be identified in more than 90% of non-AIDS patients with disseminated infection (282).

Massive histiocytic infiltration with innumerable acid-fast bacilli, resembling the "foamy" histiocytes of lepromatous leprosy, occurs in some patients and may immediately suggest the diagnosis. However, the histopathologic changes in severely immunocompromised hosts are often nonspecific with necrotizing acute and chronic inflammation, histiocytosis, and a lack of granulomatous inflammation or apparent acid-fast bacilli (150). Chronic ulceration of duodenal and colonic mucosa with histiocytic infiltration, which was ultimately fatal due to gastrointestinal hemorrhage, has been reported (337). Despite administration of multiple antimycobacterial agents, most cases of disseminated disease have been fatal (55 to 100%), particularly in children and immunocompromised hosts (230, 305, 482).

Stone et al. (441) recently presented two cases of disseminated MAC disease in children without HIV infection and reviewed an additional 30 cases of serious MAC disease in children involving visceral dissemination, localized pulmonary disease, disseminated osteomyelitis, mastoiditis, otomastoiditis, meningitis, and mediastinal mass. The overall mortality for all of the patients included in this study was 41%; however, for children with visceral dissemination, the mortality was 82% (approximately one-third of the patients had visceral disseminated disease). In contrast, patients with localized disease or osteomyelitis had a favorable outcome.

Unusual Sites of Infection

The MAC has been implicated in numerous articular and periarticular infections, causing granulomatous inflammation of any joint, bursa, or tendon sheath, but with the joint spaces of the hands and wrist most commonly involved (141, 217, 445, 499). Extension to adjacent bone and soft tissue occasionally occurs. Trauma, puncture wounds, and needle injection are common inciting risk factors, but hematogenous dissemination, particularly in patients with underlying disease, is likely. The disease is typically indolent and delays in diagnosis are common; at least 40% of the cases of NTM in one series had received intra-articular steroids for nonspecific tenosynovitis or arthritis prior to recognition of the infection (217). In only 15% of the cases can the diagnosis be made on culture of joint aspirate and surgical biopsy, and culture of synovial material is necessary for diagnosis in most cases. The majority of cases respond, with preservation of joint function, to a combination of surgical excision of infected material and antituberculous chemotherapy (141). A single case of reactive arthritis in a patient with *M. avium* pulmonary infection has been described (313).

Osteomyelitis, usually with multiple bony lesions, skeletal destruction, contiguous abscess formation, and draining sinuses, is rare (305, 482). It most commonly occurs in children who have hematologic malignancy, but rarely in apparently healthy individuals (19, 97, 259).

NTM infection of the urinary tract, which may be clinically indistinguishable from tuberculosis, is uncommon (90, 377) but can involve any structure in the genitourinary system including granulomatous prostatitis in a recently reported unusual case in an immunocompetent elderly male (336). The presence of MAC in the urine does not necessarily imply tissue infection, however.

Numerous cases of cutaneous abscesses, ulcerations, or nodules due to infection with MAC organisms have been reported, and these have been a result of either direct inoculation, trauma, or surgery or, more often, a consequence of hematogenous dissemination in an immunosuppressed patient (96, 157, 189, 307). Frank cellulitis is rare (415). Localized infection of breast tissue after breast augmentation and silicone injection due to *M. avium* has been reported (379), but *M. fortuitum* and *M. chelonae* are more commonly the cause of these mycobacterial infections.

Acute otolaryngeal, mastoid, and mediastinal infections have been described, probably as a result of extension of infection from the adjacent pharyngeal spaces (274, 285, 469). Also, there are reports of mycotic aneurysmal infection (135, 149), peritonitis associated with ambulatory peritoneal dialysis (390), and corneal ulceration (288).

CLINICAL DISEASE IN PATIENTS WITH AIDS

Focal Disease

Patients with AIDS may present with infection of the respiratory tree or gastrointestinal tract; such infection may be symptomatic or asymptomatic. Distinction between colonization and infection is often difficult, particularly in asymptomatic patients. The MAC is not uncommonly isolated from sputum or stool culture specimens in patients with AIDS (22, 202, 214, 231, 257, 388, 436), and patients may have a single positive culture of either sputum or stool or episodic excretion of organisms without apparent disease. While isolation of MAC organisms in stool is common in the absence of apparent clinical disease in HIV-infected patients without AIDS, 20 to 45% of patients with AIDS and positive stool cultures will have evidence of disseminated disease (120, 186, 388, 436). The presence of the MAC in cultures of either sputum or stool is a risk factor for disseminated disease, but approximately 64 to 75% of patients who develop bacteremia have no previous evidence of colonization (87). The detection of MAC organisms in cultures of stool or respiratory secretions in patients at risk for disseminated disease should therefore prompt a thorough search for evidence of focal or disseminated disease should therefore prompt a thorough search for evidence of focal or disseminated disease, but the routine screening of stool and sputum specimens is not advocated.

Some patients with AIDS may present with focal pulmonary infection due to *M. avium* without evidence of dissemination (342, 468). The clinical presentation is similar to that of immunocompromised hosts but is generally milder than tuberculosis (342). Patients may complain of persistent productive cough, dyspnea, fever, sweats, malaise, and weakness; hemoptysis rarely occurs. The pattern of radiographic involvement is varied. Diffuse interstitial or reticulonodular infiltrates occur in approximately 50%, alveolar infiltrates occur in 20%, and apical scarring or upper lobe involvement occurs in <10% of patients. In contrast to non-AIDS patients with pulmonary MAC infection, cavitary disease is unusual (<5%). The thick pleural reaction often seen in normal hosts with chronic pulmonary disease is not seen, and pleural effusions are rare (Fig. 3 and 4).

MAC pulmonary disease may be clinically and radiographically indistinguishable from bacterial pneumonia or pulmonary disease due to pneumocystosis, tuberculosis, aspergillosis, cryptococcosis, or coccidioidomycosis. Determination of the etiologic agent may be difficult, and more than one pathogen may be present. Despite the isolation of *M. avium* or *M. intracellulare* from cultures of sputum or bronchoalveolar lavage fluid, a careful search for other potential pathogens should be made. In a patient who has a single sputum or bronchoalveolar lavage culture positive for MAC, radiographic evidence of pulmonary infiltrative disease more likely signals the presence of a pathogen other than MAC organisms (314). Transbronchial biopsy or percutaneous needle biopsy may be necessary, but open lung biopsy should be considered in those patients in whom other measures have failed to reveal the diagnosis and in whom assessment suggests that the benefits outweigh the risks.

In the absence of data specific for patients with HIV infection, HIV-positive patients with sputum repeatedly culture positive for MAC and with persistent symptoms and/or evidence of radiographic disease, not attributable to another pathogen, may be considered candidates to receive antimycobacterial therapy. However, any HIV-positive patient with isolation of acid-fast bacilli in the sputum which has not yet been identified, particularly those with CD4 counts of >100 per mm^3 or abnormalities on chest radiographs, regardless of the results of PPD skin testing, should receive empiric therapy active against *M. tuberculosis* until the identity of the organism can be established.

Peripheral lymphadenitis due to *M. avium-M. intracellulare* occasionally occurs in patients with HIV infection without evidence of disseminated disease, sometimes associated with overlying cutaneous lesions (14). In patients with fever of undetermined etiology and negative mycobacterial blood cultures, gallium scanning may identify infected lymph nodes accessible for biopsy (12a, 308). Using the modified Diff-Quik methods, smears of fine-needle aspirate material may reveal histiocytes with negatively stained linear cytoplasmic inclusions, termed pseudogaucher cells (438). On histopathologic examination, poorly defined granulomas with histiocytes filled with mycobacteria are common; well-formed granulomas with fibrosis, necrosis, epithelioid histiocytes, lymphocyte infiltration, and Langhans' giant cells are present in less than one-third of cases (287). While lymphadenitis may occur in patients who have disseminated MAC infection, isolated peripheral node involvement is more likely due to *M. tuberculosis* (342). Patients with histopathologic evidence of granulomatous inflammation or acid-fast bacilli that have not yet been identified or do not grow in culture should probably receive empiric antituberculous therapy. It is important to point out that some cases of culture-negative disease could be caused by one of the recently recognized species of mycobacteria, *Mycobacterium haemophilum* and *Mycobacterium genavense*, which grow only under certain culture conditions (62, 105, 402).

Disseminated Infection

Disseminated infection due to the MAC in patients with AIDS commonly causes a progressive illness characterized by intermittent fever, sweats, weakness, anorexia, and weight loss; it is believed to be a major cause of wasting syndrome in patients with AIDS. Most patients, by the time they present for evaluation, will complain of 2 to 6 weeks of recurrent fever and unexplained weight loss. Approximately 40% will have nausea or diarrhea, 20% will complain of vomiting, and a few may complain of intractable, crampy abdominal pain (278, 342). On examination, hepatic and splenic enlargement is common, but significant peripheral lymphadenopathy (>1.0 cm) is unusual (<9% of cases). Possible clues to the presence of disseminated disease in a patient at risk and who has unexplained fever may be either worsening anemia or a markedly elevated alkaline phosphatase, not necessarily associated with comparable elevations in hepatic transaminases (266, 275, 279).

While the mononuclear phagocyte system is the predominant site of infection, almost any organ system can be involved, including the skin (14, 359), bone and joints (59), eyes (94, 202), thyroid (202), large airways (330, 374), adrenals (202), testis (133), and brain. Isolation of *M. avium* from the cerebrospinal fluid has been reported in patients with disseminated disease (255), but the significance of this finding is not known. Although the adrenals are commonly infected, adrenal insufficiency or a blunted response to adrenocorticotropin stimulation is more likely due to concomitant infection with cytomegalovirus (174).

Although the gastrointestinal tract may be an initial site of infection (22, 120, 231, 257, 388), patients with histologic evidence of gastrointestinal involvement invariably have disseminated disease (120, 186, 388, 436). Duodenal or rectal

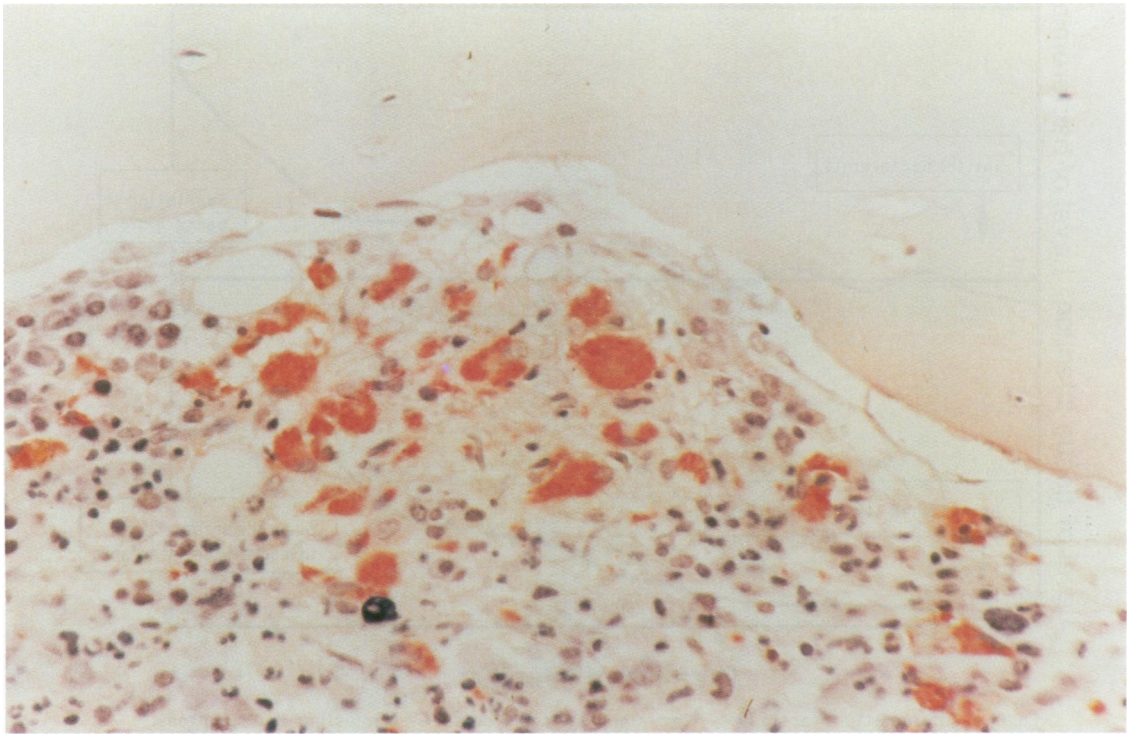

FIG. 5. Intracellular replication of MAC within fixed-tissue macrophages in a bone marrow biopsy specimen obtained from a patient with AIDS. The tissue was fixed and stained with a mixed polyclonal rabbit antimycobacterial antibody immunoperoxidase stain (478). Photomicrograph is courtesy of Stephen D. Nightingale and Elizabeth L. Wiley.

biopsies may be diagnostic. In one series, fine white mucosal nodules believed to be characteristic of MAC infection were visualized in the duodenum on endoscopy in 88% of patients with documented gastrointestinal involvement; associated malabsorption, as determined by the D-xylose test, was common (186). Colonic, sigmoid, and rectal involvement is also common, and esophageal, colonic, and rectal erosions and ulcerations due to MAC organisms may occur (120, 186, 405, 484). Upper gastrointestinal studies may reveal dilatation and thickening of the mucosal folds of the small bowel which may be clinically indistinguishable from lymphoma (463). Multiple large retroperitoneal and mesenteric lymph nodes are often demonstrated on abdominal computed tomographic scans (363). Patients may have marked histiocytic and mycobacterial infiltration on histopathologic specimens which, when visualized in the small intestine, resembles bovine paratuberculosis (Johne's disease) or Whipple's disease (185a, 287, 405, 463).

Bacteremia, with the organism found almost exclusively in circulating monocytes, occurs in 86 to 98% of patients with disseminated disease. Most patients have colony counts in the range of 10^1 to 10^3 CFU/ml of whole blood (200), but high levels of mycobacteremia, with up to 10^6 CFU/ml, are not uncommon (202, 342, 466, 485). The tissue load of infection may be 10^2 to 10^5 times greater than that in the blood. While a few patients have continuous low levels of mycobacteria in their bone marrow and bloodstream, suggesting that they have, to a limited degree, control of the infection, intracellular replication within macrophages is unchecked in many patients (Fig. 5). The large numbers of organisms within circulating monocytes and fixed-tissue macrophages, even after prolonged treatment, are evidence of an immune deficiency that profoundly impairs the ability of the host immune system to restrict the intracellular growth of these mycobacteria.

While it is not known to what degree the level of mycobacteremia correlates with the level of infection in tissues, the assessment of changes in the numbers of circulating mycobacteria has evolved as a surrogate marker of therapeutic efficacy (88, 123, 202, 278). The use of quantitative bacteremia as an endpoint in clinical trials is based on the presumption that mycobacteremia will not decrease or disappear spontaneously. While limited data suggest that the level of bacteremia progressively increases in patients who do not receive treatment (123, 257), a preliminary study suggests that the correlation between the level of infection in tissues and that in the bloodstream may be poor (479). Recent data indicate that patients who have disseminated MAC infection may have fluctuating low levels of mycobacteremia and intermittently negative blood cultures.

We identified 9 patients, including 7 of 60 patients (12%) enrolled in a prospective randomized clinical trial of MAC bacteremia (276, 277), in whom bacteremia became undetectable in the absence of antimycobacterial therapy (275). All patients had at least two negative blood cultures by both lysis-centrifugation and BACTEC methods 1 to 57 days after their first positive blood culture. Such patients reported fewer and less severe symptoms and survived longer than patients with sustained bacteremia (59 versus 31 weeks, respectively). Although the data were not statistically significant, the mean alkaline phosphatase level was lower in patients with transient bacteremia than in patients with sustained bacteremia (0.96 versus 1.68 times the upper limit of normal, respectively), and there was no apparent difference in the duration of AIDS, leukocyte count, hematocrit,

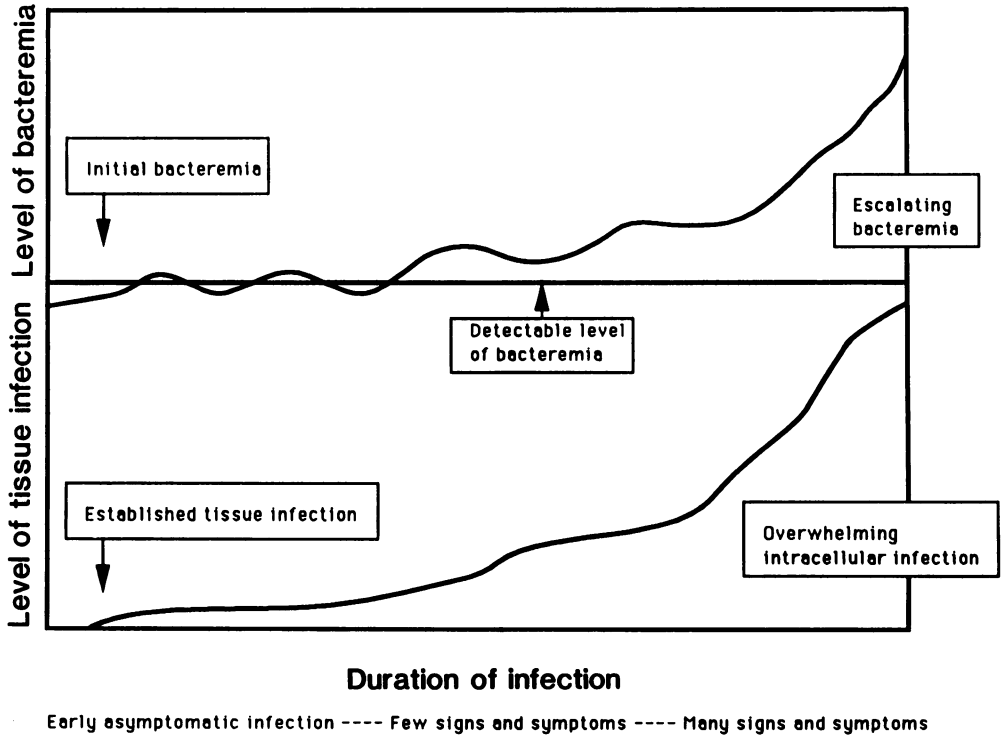

FIG. 6. Schematic representation of the course of disseminated MAC disease characterized by initial fluctuations in both mycobacteremia and clinical symptomatology but inexorably leading to a sustained and substantial mycobacteremia, most likely reflecting the underlying and often overwhelming infection of tissue.

$CD4^+$ cell count, or body weight between the two groups. Whether patients with transient bacteremia were diagnosed at an earlier stage of disease or whether they had inherently better immunity to combat infection is not known, but it is likely that these patients had less total body load of organisms than patients with sustained bacteremia. Despite the administration of one or more antimycobacterial agents for varied periods of time, six of the nine patients had subsequent recurrence of bacteremia 4 to 45 weeks after their negative pretreatment cultures, four of which occurred after treatment had been discontinued. These data suggest that these patients had established tissue sites of infection in which microorganisms were suppressed for varied periods of time but were released in transient "showers," just above the level of detection of bacteremia (Fig. 6).

Of interest, data obtained during the large rifabutin MAC prophylaxis trials (74, 181) suggest that at the time the first positive blood culture was obtained only approximately 36% of patients had self-reported fever or sweats. Approximately 30% of persons who first developed MAC bacteremia had no apparent signs or symptoms, although most became symptomatic within 1 to 2 months. Many had only one or two signs or symptoms suggestive of MAC infection, including a 5% weight loss, a decrease in hemoglobin of >1.0 g/dl, or an increase in alkaline phosphatase of >300 U/liter. Less than 30% of patients had fever (or sweats) and a 5% weight loss, and only 7% of the patients had the classic constellation of fever (or sweats), weight loss, and anemia at the time bacteremia was first detected. These data suggest that it may be difficult to recognize the presence of early infection in patients receiving prophylaxis.

Delayed Confirmation of Clinical Diagnosis

Delay in identification of acid-fast bacilli visualized by smear from respiratory secretions, tissue specimens, or stool smears is particularly problematic in the management of AIDS patients. While several clues may facilitate the management of a patient with an infection due to an unidentified mycobacterium, because of the fulminant nature of tuberculosis in patients with AIDS, the availability of effective therapies, and the public health implications of an untreated infection, the possibility of *M. tuberculosis* infection requires a guarded and conservative approach (Table 1). Empiric antituberculous therapy is probably warranted for those patients who have isolated peripheral lymphadenitis or clinical and radiographic pulmonary disease and culture or smear evidence of infection due to an unidentified mycobacterium. The presence of a positive blood culture for mycobacteria in these circumstances, however, makes the diagnosis of tuberculosis somewhat less likely. Blood cultures are positive in 86 to 98% of patients with disseminated MAC, often within 14 days (reflecting the high level of bacteremia), but are rarely positive in patients with tuberculosis (202, 342, 466, 485). While sputum smears are much more likely to be positive in patients with tuberculosis than in those with MAC infection (83 versus 16%), both organisms are isolated with fairly equal frequency from lymph node, bone marrow, and stool specimens (342). The frequency of tuberculosis also depends, to some degree, on the patient's sex, ethnicity, and HIV risk group.

TABLE 1. Features which may distinguish between MAC and *M. tuberculosis* (MTB) infection in HIV-infected patients also infected with an unidentified mycobacterium[a]

Feature	MAC patients	MTB patients
Sex, ethnicity, HIV risk group	More likely non-Hispanic, white homosexual male	More likely African-American, Hispanic, Haitian, female, or intravenous drug user
AIDS status	>90% have preexisting AIDS	70% do not have AIDS
CD4 counts	Rarely >100/mm^3	Any level of immunity
Chest radiograph	Usually normal (75%)	Frequently abnormal (83%)
Pulmonary disease	Unusual, 4–10%	Occurs in 70%
Pulmonary findings	Unusual to have hilar lymphadenopathy, cavitary disease, or pleural effusions	25% with hilar lymphadenopathy; cavitary disease and pleural effusions may occur
Sputum	16% of smears positive; 25% of cultures positive	60% of smears positive; 70% of cultures positive
Extrapulmonary disease	Common	Common
Bacteremia	>85% of patients	2–12% of patients
Blood cultures	Positive in 1–4 wk	Positive in 4–8 wk
Stool	40–50% of smears and cultures positive	40–50% of smears and cultures positive

[a] Compiled, in part, from several sources (233, 245, 342, 468).

In addition to these findings, the onset of mycobacterial disease relative to the onset of AIDS may be helpful in distinguishing between tuberculosis and MAC disease. Tuberculosis occurs at any level of immunity, characteristically precedes the diagnosis of AIDS in 40 to 67% of cases, and occurs as the AIDS-defining illness or concurrent with AIDS in 26% of cases (342, 433). In contrast, only 3 to 13% of MAC infections represent the AIDS-defining illness, frequently concurrent with another opportunistic infection, and the majority of cases usually occur late in the course of AIDS (199, 234, 358). Differentiation between the two infections may be more difficult in patients with severely depressed immunity. While the presentation of tuberculosis in patients with HIV infection and relatively intact immunity is similar to that of non-HIV-infected patients, patients with severely depressed CD4$^+$ cell counts often present with atypical radiographic features, a lack of cavitary disease or lymphadenopathy, and a greater incidence of extrapulmonary disease (95). Despite a high incidence of anergy, skin test reactivity should be determined and, when negative, repeated in 1 to 4 weeks. Credence should not be given to a negative skin test with PPD in a patient strongly suspected of having tuberculosis.

THERAPEUTIC AGENTS AND TREATMENT

Licensed Therapeutic Agents

The agents most commonly used in the treatment of infection due to MAC include parenterally administered amikacin and orally administered clofazimine, ciprofloxacin, ethambutol, isoniazid, rifampin, and rifabutin. In clinical trials in patients with AIDS, two recently introduced macrolides, clarithromycin and azithromycin, demonstrated remarkably impressive bacteriologic activity. The therapeutic dosages and adverse side effects of these agents are addressed in Table 2.

Amikacin. Amikacin, a semisynthetic aminoglycoside antibiotic derived from kanamycin, remains one of the most bactericidal agents against MAC both in vitro and in the beige mouse model (49, 161, 165, 246). Analysis of in vitro susceptibilities of clinical isolates indicates that 9% of MAC isolates are susceptible to 12 μg of amikacin per ml, but 75% are susceptible to 30 μg/ml (317). In the beige mouse model, administration of amikacin resulted in 1.2- and 2.6-log$_{10}$ reductions in splenic and pulmonary CFU per milliliter, respectively, by 2 weeks (165). The addition of clofazimine to amikacin also was effective, resulting in a more than 4-log$_{10}$ reduction in colony counts in spleen tissue, but the addition of rifabutin did not appear to enhance the microbiologic activity of the two-drug combination. Unfortunately, amikacin is not absorbed from the gastrointestinal tract and requires parenteral administration, usually in a single or divided dose of 7.5 to 15 mg/kg of body weight per day. The most significant adverse effects are ototoxicity and nephrotoxicity, and ototoxicity may develop in up to 13% of patients with AIDS (23, 278). Some of these dose-related toxicities may be ameliorated by lower dosages and a shorter total course of administration.

Azithromycin and clarithromycin . The macrolide antibiotics azithromycin and clarithromycin are similar in structure to erythromycin (384) and concentrate to high levels in tissues and macrophages with little toxicity. Clarithromycin differs by a single substitution of a methyl group for a 6-hydroxyl group in the 14-membered ring of erythromycin. This substitution increases its bioavailability, decreases metabolism of the drug, and enhances the microbiologic activity. Clarithromycin is resistant to the intramolecular cyclization at acidic pH; thus it lacks much of the gastrointestinal side effects commonly observed with erythromycin. Clarithromycin is metabolized in the liver to 14-OH-clarithromycin, which is biologically active against many microorganisms and partially active against MAC. Clarithromycin is rapidly absorbed, with a bioavailability of approximately 55%; peak blood levels of 2 to 3 μg/ml are seen 2 h after a 500-mg dose. The serum half-life after a 500-mg dose is 5 to 6 h, while that of 14-OH-clarithromycin is 7 h.

Clarithromycin inhibited more than 90% of MAC strains at concentrations that are therapeutically achievable in humans (153, 350). The MICs, as determined by broth microdilution at neutral to slightly alkaline pH, were 0.25 to 0.5 μg/ml for most strains (212, 378). Administration of clarithromycin to beige mice resulted in a significant reduction in the number of mycobacteria in tissue and blood (153). The intracellular activity of clarithromycin in J774 cells and in alveolar macrophages from HIV-infected patients was enhanced by the addition of ethambutol and rifampin, but the addition of ciprofloxacin did not improve intracellular killing (493). The activity of this three-drug regimen (clarithromycin, etham-

TABLE 2. Antimycobacterial agents commonly used in the treatment of MAC infection[a]

Agent	Adult dose	Pediatric dose[b]	Adverse effects
Common agents			
Amikacin	7.5–15 mg/kg QD[c]	10–15 mg/kg QD i.v.[c]	Ototoxicity, nephrotoxicity
Azithromycin	500 mg/day[d]	10–20 mg/kg/day[e]	Diarrhea, nausea, vomiting, abdominal pain, headache, dizziness, elevations in hepatic enzymes
Ciprofloxacin	750 mg BID	20–30 mg/kg/day, divided, Q12h[f]	Anorexia, nausea, vomiting, abdominal pain, diarrhea, rash, (rarely) mental status changes
Clarithromycin	500–1,000 mg BID[g]	30 mg/kg/day, divided, Q12h[e]	Diarrhea, nausea, vomiting, elevations in hepatic enzymes, abdominal pain, renal insufficiency
Clofazimine	100–200 mg/day[h]	1–2 mg/kg/day[i]	Skin discoloration, ichthyosis, anorexia, nausea, vomiting, abdominal pain, peripheral neuropathy, (rarely) ocular changes
Ethambutol	15 mg/kg/day[j]	15–25 mg/kg/day[k]	Anorexia, nausea, vomiting, diarrhea, rash, mental status changes, retrobulbar neuritis
Rifampin	10 mg/kg/day[l]	10–20 mg/kg/day[m]	Anorexia, nausea, vomiting, diarrhea, rash, elevations in hepatic enzymes
Rifabutin	300 mg/day[n]	No recommendation[n]	Anorexia, nausea, vomiting, diarrhea, rash, myalgias, arthralgias, headache
Alternative agents			
Cycloserine	10–20 mg/kg/day	10–20 mg/kg/day	Somnolence, headache, tremor, vertigo, mental status changes, visual changes, seizures
Ethionamide	15–20 mg/kg/day	15–20 mg/kg/day	Anorexia, nausea, vomiting, diarrhea, rash, elevations in hepatic enzymes, mental status changes, seizures, neuropathy

[a] QD, once a day; i.v., intravenously; BID, twice a day; Q12h, every 12 h.
[b] Pediatric dosages are not to exceed maximum adult dosages.
[c] Or equivalent dose twice a day.
[d] Dosages of 600 to 1,200 mg/day in a lactose-free formulation are being studied in a dose-ranging fashion.
[e] Pediatric suspension formulations are available for both azithromycin and clarithromycin; azithromycin maximum dose, 40 mg/kg.
[f] Ciprofloxacin is not recommended for children under 18 years of age; however, ciprofloxacin and other quinolones have been, when necessary, administered to children with few serious adverse effects (418).
[g] Dosages of up to 2,000 mg orally twice a day have been used in the treatment of MAC infection but are associated with higher rates of toxicity and should probably be reserved for those patients failing to respond to lower dosages.
[h] Dosages of up to 300 mg/day have been used in the treatment of other diseases and can be administered to patients with AIDS and MAC bacteremia but are associated with a higher incidence of skin discoloration and gastrointestinal toxicity.
[i] Dosages of up to 50 mg/day have been given to children less than 4 years of age (approximately 4 mg/kg/day) (297), but a pediatric formulation is not available.
[j] Dosages of up to 25 mg/kg/day can be given for short durations (<1 month); dose should not exceed 600 to 1,000 mg/day.
[k] Caution is recommended in children under 12 years of age; monthly vision checks should be performed on pediatric patients receiving ethambutol or adults receiving >15 mg/kg/day for more than 1 month. The maximum dose is 2.5 g.
[l] Dosages of 150 to 600 mg/day have been used in both AIDS and non-AIDS patients with MAC infection, but the relative efficacies of these various dosages are not known.
[m] The maximum rifampin dose is 600 mg/day.
[n] Dosages of 75 mg/day have been given to children less than 4 years of age (approximately 6 mg/kg/day) (297), but a pediatric formulation is not available. Higher dosages up to 150 mg/day (6 to 25 mg/kg/day) have been used (301).

butol, and rifampin) has been confirmed in other in vitro analyses (439). In one small trial, the combination of clofazimine and clarithromycin resulted in clearance of bacteremia in all 11 patients after only 1 week of therapy (409). In another recent clinical trial in patients with AIDS, 75% of patients developed negative blood cultures after 1 to 2 months of single-agent therapy (123). After administration for more than 10 to 22 weeks, however, drug resistance and rebound bacteremia were seen in some patients. Clarithromycin administered in a dose of 500 to 1,000 mg twice daily is moderately well tolerated. While adverse effects are reported in approximately 4% of patients (384), gastrointestinal side effects are apparently more common in patients with AIDS.

Azithromycin, an azalide, has an additional nitrogen in the erythromycin ring structure, resulting in a 15-member derivative. The drug is well absorbed and has a terminal half-life of 68 h. Peak serum concentrations after single or multiple 500-mg doses range from 0.40 to 0.62 μg/ml, but the drug concentrates within macrophages and in tissues to remarkably high concentrations, as high as 2,000 μg/g (49, 172, 173). The in vitro activity of azithromycin appears modest, with a broad range of MICs from 32- to 64-fold above the peak serum concentration in humans. Nevertheless, in beige mice, azithromycin had significant activity against the MAC, resulting in a 95% survival and a significant decrease in the number of mycobacteria in blood, liver, and spleen (247). The therapeutic efficacy most likely reflects the high tissue

concentrations of the drug. A recent uncontrolled pilot trial showed that, when patients with AIDS and MAC bacteremia received 20 to 30 days of azithromycin alone (500 mg/day), colony counts in the bloodstream were reduced from 2×10^3 to 1.4×10^2 CFU/ml; fever resolved in 71% of patients who were febrile at entry to the study and sweats resolved in 83% (505). Long-term administration of azithromycin resulted in recrudescence of bacteremia and high-level resistance. Thus, the administration of azithromycin or clarithromycin as a single agent is not recommended. Adverse reactions reportedly occur in less than 12% of patients and include diarrhea (3.6%), nausea (2.6%), and abdominal pain (2.5%), as well as headaches and dizziness (1.3%) (384), but gastrointestinal complaints appear more common in patients with AIDS. Deafness has been reported in a small number of patients during long-term administration but is apparently reversible upon discontinuation of the drug.

Clofazimine. Clofazimine is an iminophenazine red dye with a long elimination half-life; the elimination half-life from fatty tissues and the mononuclear phagocyte system is approximately 70 days (167); the drug is highly concentrated in tissues. Clofazimine has been a mainstay of leprosy therapy. The drug is very active (on a weight basis) in vitro against most MAC isolates; the MIC for 90% of *M. avium* isolates (MIC_{90}) is approximately 3 μg/ml and that for *M. intracellulare* isolates is approximately 2 μg/ml (306, 452). In the beige mouse model, clofazimine is extremely effective in combination with amikacin (165); however, in patients with AIDS and MAC bacteremia, clofazimine, administered as a single agent (200 mg/day), resulted in a median reduction of only 0.20 $\log_{10}$ CFU/ml in the bloodstream (18% reduction in baseline colony counts) by 4 weeks (277). These data suggest that effective clofazimine therapy may require longer periods of treatment to saturate the tissues; e.g., in patients with leprosy, it may take up to 4 months to achieve a 99% reduction in tissue colony counts. Clofazimine is administered in a 100- to 200-mg daily dose (up to 300-mg daily doses have been used in the treatment of other diseases), and the drug is fairly well tolerated, with skin discoloration as the most frequent side effect. Dose-limiting intolerance occurs in approximately 2.5% of patients with AIDS (278), but the skin discoloration is more common at dosages of ≥200 mg/day. Clofazimine crystals may be deposited in organs and cause intractable abdominal pain, a symptom often confused with MAC infection (167, 239, 416). While clofazimine crystals are found in the tears of 32% of leprosy patients receiving long-term therapy (273), ocular effects, such as corneal-conjunctival pigmentation and a "bull's-eye" retinopathy, are unusual (112, 273, 429). Clofazimine treatment also has been associated with peripheral neuropathy.

Ciprofloxacin. Ciprofloxacin and the other fluoroquinolones (e.g., ofloxacin, levofloxacin, lomefloxacin, sparfloxacin, and WIN 57273) have varied in vitro activities against the MAC (211, 246, 303, 503). MIC_{50} and MIC_{90} values of ciprofloxacin against *M. avium*, as determined by broth dilution susceptibility tests on large numbers of isolates, are 4 and 16 μg/ml, and those against *M. intracellulare* are 1 and 8 μg/ml, respectively (303). Only 30% of MAC isolates were susceptible to 2 mg of ciprofloxacin per ml. Values for ofloxacin were similar. Quinolone resistance is common and related, in part, to the mechanism of action (inhibition of DNA synthesis). Ciprofloxacin is administered in a dosage of 750 mg twice daily and is moderately well tolerated. Dose-limiting side effects, primarily gastrointestinal, occur in up to 15% of patients with AIDS (278); rash, headaches, and mental status changes may occur. Ciprofloxacin should not be administered in conjunction with magnesium- or aluminum-containing antacids or sucralfate. Absorption of ciprofloxacin is effectively negligible after ingestion of 2.0 g of sucralfate. Ciprofloxacin inhibits the metabolism of methylzanthines, including theophylline, and there is an intriguing, but preliminary, observation that quinolones may induce IL-2 production and decrease IL-2 receptor expression, resulting in prolonged IL-2 kinetics (398).

Ethambutol. Ethambutol is a dextro-2,2′-(ethylenediimino)-di-1-butanol-dihydrochloride with a high degree of antituberculous activity. A recent analysis demonstrated that only 7% of MAC isolates tested were susceptible to 5 μg of ethambutol per ml, but 76% of isolates were susceptible to 10 μg/ml (317). Although these susceptibility tests suggest that ethambutol should not be a very effective agent, ethambutol may potentiate the action of combined therapies as a result of the effect of this drug on cell wall permeability (220, 268, 490). Nevertheless, recent animal and human studies indicate that ethambutol alone has significant anti-MAC activity. In one study, ethambutol reduced colony counts in beige mice in a dose-response fashion; at 6 mg/kg per day, mycobacteria were reduced by approximately 1.0 $\log_{10}$ by 9 weeks (183). Furthermore, ethambutol (15 mg/kg/day), administered as a single agent, significantly reduced mycobacteremia by a median 0.6 $\log_{10}$ CFU/ml after 4 weeks in patients with AIDS and MAC bacteremia (276). Ethambutol is commonly administered in a dose of 15 mg/kg of body weight per day, usually as a single dose, and is fairly well tolerated in the treatment of MAC disease. Dose-limiting side effects, primarily gastrointestinal, may occur in 5 to 10% of patients with AIDS, but severe toxicity is unusual (278, 433). Higher doses (25 mg/kg of body weight) have been used, but may be associated with retrobulbar neuritis and loss of color vision. These side effects are uncommon, typically associated with long-term use (longer than 1 month), and in most cases, reversible if the drug is promptly discontinued.

Rifampin. Rifampin is a 3,4-(methylpiperazinyl-iminomethylidene)-rifamycin SV and is in the rifamycin group of antimicrobial agents. Rifampin is a broad-spectrum antimicrobial agent with antituberculosis activity but only modest anti-MAC activity. The concentration of rifampin in tissues is significantly higher than that in serum, and rifampin is concentrated fourfold above serum levels in mouse macrophages and fivefold above serum levels in human monocytes (410). The in vitro activity of rifampin is heterogeneous, with significant differences between the various MAC serovars (452), but most MAC isolates are resistant in vitro, with rifampin MICs of >100 μg/ml (317). The activity of rifampin in combination with other agents is not known, but in vitro data indicate that some combinations are synergistic (490). In patients with AIDS and MAC bacteremia, logarithmic colony counts in the bloodstream actually increased 17% after 4 weeks of rifampin alone (276). The commonly administered dose for the treatment of MAC disease is 600 mg/day as a single or divided dose for patients weighing 50 kg or more (typically 10 mg/kg of body weight). It is well absorbed when taken without food (patients should be advised to take rifampin at least 2 h before or after any meal), and a peak serum concentration of approximately 10 mg/ml occurs within 2 h of oral administration. Rifampin is moderately well tolerated, but approximately 12% of patients with AIDS will have adverse effects, usually gastrointestinal, necessitating discontinuation of the drug (278, 433).

Rifabutin. Rifabutin, an ansamycin derived from rifamycin-S, has significantly better in vitro activity against the

TABLE 3. Microbiologic efficacy of antimycobacterial agents in combination and alone

Agent(s) and daily dose	Total no. of patients (no. evaluable)	Baseline (log_{10} CFU of bacteremia/ml)	Change from baseline [log (%)][a]	No. (%) of negative cultures[b]	Reference(s)
Amikacin 15 mg/kg Ciprofloxacin 1,500 mg Ethambutol 15 mg/kg Rifampin 10 mg/kg	17	2.7	−1.5 (−56)[c]	3 (18)	88
Ciprofloxacin 1,500 mg Clofazimine 100–200 mg Ethambutol 15 mg/kg Rifampin 10 mg/kg	41 (31)	2.1	−1.4 (−80)[d]	13 (42)	278
Ethambutol 15 mg/kg	19 (15)	1.1	−0.4 (−37)[d]	1 (7)	276
Clofazimine 200 mg	21 (18)	1.7	−0.1 (−18)[d]	0 (0)	
Rifampin 10 mg/kg	20 (17)	1.4	+0.4 (+17)[d]	1 (6)	
Azithromycin 500 mg	21 (21)	3.3	−1.2 (−36)[e]	NA[f]	505
Azithromycin 600–1,200 mg	65 (41)	2.0	−1.7 (−91)[d]	19 (46)	480
Clarithromycin[g]					79, 80
500–2,000 mg BID	72 (54)	NA	NA	43 (60)	
1,000 mg BID	NA	2.8	−2.4 (−86)	NA	
2,000 mg BID	NA	2.6	−2.3 (−88)	NA	
Liposomal gentamicin 1.7–5.1 mg/kg	21 (14)	2.8	−0.6 (−22)[d]	0 (0)	357

[a] Log_{10} CFU per milliliter; percent change in CFU per milliliter is given in parentheses.
[b] Blood culture negative after 6 to 12 weeks of treatment or last patient observation if discontinued earlier.
[c] Based on change in mean logarithmic colony counts calculated at day 28 (88).
[d] Based on mean change in individual logarithmic colony counts in evaluable patients; calculated at day 28 (276, 278) or days 39 to 42 (357, 480).
[e] Based on change in mean arithmetic colony counts; calculated at days 20 to 30 (505).
[f] NA, not available.
[g] Analysis of an intent to treat.

MAC compared with rifampin (410), but in numerous studies of rifabutin as part of various combination regimens against MAC disease, there was only modest efficacy (4, 235, 364). The contribution of rifabutin to these various regimens is not known, and the dosages were variable and may not have been adequate. The elimination half-life of rifabutin after oral administration is approximately 16 h (366, 432). Although the levels of this drug in serum are low (approximately one-tenth that of rifampin), rifabutin is concentrated 5 to 10 times above the serum concentration in tissues and 15 times above the serum level in human monocytes (410). Only 13% of isolates with moderate levels of resistance to rifampin are resistant to rifabutin, but strains that are highly resistant to rifampin are cross-resistant to rifabutin (208, 213). In a large placebo-controlled preventive treatment trial in patients with AIDS, rifabutin was fairly well tolerated compared with placebo. There was, however, an increased incidence of rash, arthralgias, myalgias, and neutropenia in patients who received active drug (74, 181, 444). Severe side effects, including hepatotoxicity, were unusual. The usual dose administered to patients with MAC is 300 mg/day. Dosages of 600 to 1,200 mg/day have been used but are associated with a higher frequency of gastrointestinal effects, headache, and myalgias (63). Rifabutin has been demonstrated to increase the clearance of zidovudine in patients with AIDS by up to 43%, but the clinical significance of this finding is not known (353).

Dapsone and sulfamethoxazole. Dapsone (164, 177) and sulfamethoxazole (168) have minimal antimycobacterial activity in vitro and may contribute to reductions in mycobacteremia in certain patients. In addition, there has been renewed interest in the minimal antimycobacterial activity seen with some beta-lactam antibiotics (78, 117, 360, 491).

Isoniazid and pyrazinamide. Isoniazid and pyrazinamide are inactive in vitro against a majority of MAC isolates (244), and neither of these drugs has a role in the treatment of MAC

disease. There is little information about the use of *p*-aminosalicylic acid and capreomycin for treating MAC disease; however, these agents are not likely to be useful because of poor in vitro activity (244).

Investigational Therapeutic Agents

Newer investigational agents may prove of value in the treatment of MAC disease, but controlled clinical data are limited. Theoretically, liposomal encapsulation or similar lipid associates of a variety of antimycobacterial agents allow the delivery of high concentrations of drug to organs with the greatest burden of infection, saturating the mononuclear phagocyte system, thereby enhancing therapeutic efficacy and reducing drug toxicity. Although it is not known how liposomes deliver antimycobacterial agents to intracellular pathogens, liposomal encapsulation does enhance the activity of amikacin and rifampin in the beige mouse model (118, 139, 412). Colony counts were substantially reduced in the liver, spleen, and kidneys but only modestly reduced in the lungs compared with "free" drug. Initial dose range studies of liposome-encapsulated gentamicin in 21 patients with AIDS and MAC bacteremia demonstrated reversible nephrotoxicity after seven doses in one patient given 5.1 mg of drug per kg twice weekly (357). Colony counts in the bloodstream were variably reduced in 10 of 14 patients given doses of 1.7 to 5.1 mg/kg twice weekly for 4 weeks but were increased in three patients and were unchanged in one patient. Other liposome-encapsulated agents that have been evaluated include clofazimine, clofazimine analogs, and streptomycin. Adjuvant administration of immunomodulatory agents (e.g., GM-CSF and alpha interferon) may prove beneficial in select patients (48), but additional information is needed.

Treatment of Disease in Patients without AIDS

Subacute lymphadenitis. Treatment of lymphadenitis by surgical excision has been the standard of care for years and is usually curative. Nonexcisional biopsy should be avoided because of the increased risk of sinus tract formation. In addition to being of diagnostic value, needle aspiration may also be curative in select cases; in one small study, 9 of 17 cases responded to needle aspiration alone (8). This may be particularly helpful when nodal involvement overlies a facial nerve. Antimycobacterial therapy is seldom necessary in the treatment of lymphadenitis, except in unusual cases of extensive disease or underlying immunodeficiency (170, 284, 297, 311, 312, 423). However, the impact of the newer macrolides on nontuberculous mycobacterial lymphadenitis in children is unknown; effective chemotherapy, if available, may obviate the need for lymph node excision. Lymphadenitis due to *M. tuberculosis* must be treated with antimycobacterial agents.

Pulmonary disease. While pulmonary infection due to MAC was previously believed to be relatively benign (404), probably because of the large numbers of transiently colonized patients, recent data suggest that chronic pulmonary infection, even in those without underlying pulmonary disease, can result in significant morbidity and mortality. In one recent study, despite prolonged treatment, 4 of 21 patients (19%) eventually succumbed to overwhelming pulmonary disease (389). In another, 2 of 15 (13%) patients with asymptomatic disease and 15 of 49 (31%) patients with symptomatic disease eventually died of causes related to their MAC infection (240). Multiple-drug regimens administered for long periods of time, with surgical resection of disease in select patients, has been the main approach to this disease for years, but relapses, as defined by the recurrence of clinical symptoms and/or positive respiratory secretions, are frequent (145, 240, 389).

Sputum conversion has been correlated with the number of agents administered; thus, many clinicians advocate the use of four to six antimycobacterial agents (6, 145, 389). In one study, four of four patients treated with only two agents relapsed, whereas three of five patients treated with three drugs and only one of four patients treated with four agents relapsed (389). Most studies advocated the use of isoniazid (INH), rifampin, and ethambutol (6) with or without streptomycin, but many experts believe that INH no longer has a role in MAC pulmonary disease. The optimal duration of treatment is not known, but the usual recommended length of therapy is 18 to 24 months or at least 12 months after sputum conversion. Acid-fast smears and cultures should be obtained monthly to assess the response to treatment, and therapy should be adjusted when patients are persistently culture positive. Patients with persistently positive cultures, but with disease limited to one lobe, should be considered for surgical resection when appropriate. Several studies suggested that the prognosis is much better for patients who undergo resection of infected lung tissue (104), but complications are frequent, particularly in patients with disease in both lobes or evidence of submucosal endobronchitis at the margin of resection. Unfortunately, many patients with biapical MAC disease are not good surgical candidates because of advanced age. New active agents such as the fluoroquinolones and macrolides may improve the outlook for many of these patients.

Disseminated disease in non-AIDS patients. Although rare, disseminated MAC disease in immunocompromised patients without HIV infection is associated with a high degree of mortality, and again resistance to antimycobacterial therapy is common (230, 305, 441, 482, 483). Therapies that prove effective in the treatment of MAC disease in HIV-infected patients are likely to be effective in these other immunocompromised hosts.

Treatment of Disease in Patients with AIDS

Disseminated MAC infection is not immediately life-threatening to patients with AIDS but can severely limit their quality of life and long-term survival. While some authors initially argued that MAC was not the proximate cause of death in most patients with AIDS (289), at least two case-controlled studies identified MAC as an independent risk factor for early mortality (82, 228), as did a longitudinal study (257). The difference in median survival of AIDS patients with disseminated MAC compared with uninfected controls was approximately 7 months (4 versus 11 months) (228). Furthermore, prolonged survival was associated with antimycobacterial treatment; treated patients lived a median of 8 months (228). However, no single agent or combination of agents has proven uniformly effective in treating MAC disease in patients with AIDS, but several trials have demonstrated some degree of clinical and microbiologic efficacy (4, 12, 23, 88, 123, 182, 229, 278, 317, 466, 505) (Table 3).

In one of the earliest treatment trials of MAC bacteremia in AIDS, Hoy et al. (235) showed that in 23 of 25 patients given a combination of rifabutin, clofazimine, ethambutol, and INH, mycobacteremia was cured; however, four of these patients had a recurrence of bacteremia. Agins et al. (4), using similar dosages of the same four agents, cleared

the bacteremia in six of seven patients, but one of the patients had a persistently positive bone marrow culture and six of the patients died within 6 to 11 weeks of therapy. Both of these studies focused on subsets of patients who tolerated these four agents for 30 or more days.

Quantitative blood cultures were first used in the mid-1980s as a marker of microbiologic efficacy (202, 485). In these studies, mycobacteria were cleared from the blood of only two patients with varied combinations of clofazimine, ethambutol, ethionamide, rifampin, rifabutin, and INH. The relationship between the level of mycobacteremia and clinical response was further investigated in two larger open trials, the first of which used a combination of amikacin, ciprofloxacin, ethambutol, and rifampin (88). In 17 patients, the mean colony count decreased from 2.7 to 1.2 $\log_{10}$ CFU/ml after 4 weeks of therapy; 10 of 14 patients became afebrile. Of the 10 patients who completed 12 weeks of therapy, only 3 had no evidence of bacteremia, but there was a sustained suppression of the bacteremia (mean colony count, 0.1 $\log_{10}$ CFU/ml) in 8 patients who received more than 12 weeks of therapy. When clofazimine was substituted for amikacin, colony counts were reduced by means of 1.4 and 2.1 $\log_{10}$ CFU/ml after 4 and 12 weeks of therapy, respectively (278). Of 31 evaluable patients, 13 (42%) became culture negative by 12 weeks. Symptoms improved in more than 70% of patients who tolerated the regimen, but a clinical response was not seen until 3 or 4 weeks of therapy. Approximately two-thirds of the patients with persistent or recurrent fever after 4 weeks of therapy had evidence of another infection, suggesting that persistent symptoms were more likely due to another infection than to a failure of MAC therapy. The majority of patients continued to lose weight, suggesting a lack of efficacy or the development of complications such as TNF-α-associated cachexia or concurrent infection. Similar clinical results were obtained in a second noncomparative trial with the same combination of agents (182); preliminary results indicated that the concentrations of various agents in serum were lower than expected in a majority of patients.

In several microbiological efficacy trials, including trials of azithromycin, clarithromycin, rifampin, ethambutol, clofazimine, and sparfloxacin as single agents, quantitative changes in mycobacteremia were used as a microbiologic endpoint (Table 3). Although the statistical analyses varied, a comparison of the effect of these agents alone and in combination on MAC mycobacteremia is revealing. While the four-drug combination regimens resulted in a 56 to 80% reduction in baseline counts, the activities of the individual components of these regimens did not fully account for the reduction in counts associated with the combination (276, 278). Reductions in colony counts due to rifampin, clofazimine, and ethambutol, given as single agents, were +17, −18, and −37%, respectively. In contrast, data from a randomized, open trial of azithromycin, given as a single agent in dosages of 600 to 1,200 mg/day, showed a 91% reduction in colony counts after 6 weeks of therapy (480). Of 41 patients, 19 (46%) had negative blood cultures with azithromycin alone. In another study, after 12 weeks of clarithromycin (1,000 mg/day) therapy alone, there was a 99.5% median reduction in colony counts (79, 80); the median time to sterilization of blood cultures was 43 days. In contrast, sparfloxacin as a single agent had little effect on colony counts (506). Preliminary data with liposome-encapsulated gentamicin suggest a modest microbiologic effect (357).

Three small studies assessed the microbiologic and clinical effects of clarithromycin in combination with other agents (123, 128, 409). Bacteremia was rapidly eliminated in 11 of 11 patients who received a combination of clarithromycin and clofazimine after only 1 week of therapy (409). Using a combination of clarithromycin, ciprofloxacin, and amikacin, de Lalla et al. demonstrated clearance of bacteremia in 12 of 12 patients after 2 to 8 weeks of therapy (128). Dautzenberg et al. (123) demonstrated that bacteriologic failure occurred in 29% of patients who received less than 1,000 mg of clarithromycin per day, but failure occurred in only 3% of patients who received 1,500 to 2,000 mg/day (121, 122); however, many of these patients were receiving a variety of other agents.

On the basis of the available in vitro and in vivo data, a combination of three agents, including a macrolide, ethambutol, and a third agent (rifampin, rifabutin, ciprofloxacin, or another quinolone), may afford the best therapy for disseminated MAC infection. However, this recommendation is made in the absence of any controlled data. A U.S. Public Health Service Task Force has recommended that treatment regimens in patients with AIDS include a minimum of two agents, including one of the macrolides (461). The individualized selection of therapy is often tempered by patient considerations and tolerance. Amikacin, although extremely effective, might be reserved for those patients who are failing or intolerant of oral therapies. Some authors previously advocated a more aggressive "induction" regimen with four to six antimycobacterial agents followed by a less aggressive "maintenance" regimen of two to three agents (502); however, given the lack of urgency in most cases, the slow therapeutic response, and the high incidence of adverse effects, incremental introduction of several agents over a 1- to 2-week period is reasonable and may result in greater compliance. Zidovudine therapy should be continued whenever possible, since antiretroviral therapy has been associated with a clinical and microbiologic improvement in patients with disseminated MAC (17).

While the results of in vitro susceptibility and quantitative blood culture studies may be helpful in guiding the selection of therapy, often this information is not available when a therapeutic decision is clinically required. Many clinicians offer empiric antimycobacterial therapy when faced with a febrile patient at risk for disseminated MAC infection. This approach is reasonable provided other causes of fever have been eliminated, adequate pretreatment cultures are obtained, and the need for continued therapy is periodically reevaluated. Susceptibility studies may provide helpful information when a patient develops a recurrent or persistent bacteremia and resistance is suspected. This is particularly true with regard to the macrolides, to which resistance is well documented; however, it is important to point out that there is no consensus about susceptibility test methodology or interpretive criteria.

The optimal duration of treatment for disseminated MAC infection is unknown, but discontinuation of therapy often results in a recurrence of bacteremia (275, 278, 505); however, there may be a window of several weeks to months before the bacteremia returns (275). Despite prolonged and presumably effective treatment, many patients die with evidence of MAC infection (278); therefore, therapy should be continued indefinitely or, at a minimum, for several months after blood cultures become negative.

Children with HIV infection and disseminated MAC present with signs and symptoms similar to those of adults with HIV infection (73, 242). Lewis et al. (302) recently described 21 children with AIDS and disseminated MAC

(one adolescent had localized lymphadenitis) disease. Overall, 11% of the 196 patients included in the study developed MAC disease, but this value increased to 24% in patients with CD4 counts of <100 per mm^3. The patients with MAC disease had higher p24 antigen levels; however, there was no significant difference in the survival of children with (46 weeks) or without (50 weeks) MAC disease. While there are little data on the optimal management of pediatric patients, combinations of various antimycobacterial agents have been administered with modest clinical and microbiologic success. Almost all of the antimycobacterial agents used to treat adults can be administered to pediatric patients, but little data are available on the dosing and safety of many agents. Clofazimine, 50 mg/day (approximately 4 mg/kg/day), has been successfully and safely administered to two children less than 4 years of age, but this regimen requires splitting the capsule (297). Rifabutin has been safely administered to a small number of children in a dose of 75 mg/day (approximately 6 mg/kg/day) (297). Ethambutol has been administered to pediatric patients, but children may be more likely to develop adverse ocular effects compared with adults, particularly at the higher dose (Table 2). Both azithromycin and clarithromycin are promising agents for treating MAC disease in children, and both drugs are now available in liquid formulations. Preliminary results obtained in 20 pediatric patients treated with clarithromycin as a single agent in doses ranging from 7.5 to 30 mg/kg/day suggest that this agent is both safe and moderately efficacious, particularly at 30 mg/kg/day (241).

Prophylaxis

Prevention or delay in bacteremia was demonstrated in two large (and almost identical), concurrent, double-blind, placebo-controlled trials in patients who had AIDS and $CD4^+$ cell counts of <200 per mm^3. Patients were treated with either rifabutin, 300 mg orally daily, or placebo for a mean duration of 7.4 months (74, 181). Combining the intent-to-treat analyses from both studies, 48 of 566 (8.5%) patients who received drug developed at least one positive blood culture for MAC bacteremia compared with 102 of 580 (17.6%) patients who received placebo. Overall, the frequency of MAC bacteremia was reduced by about 50% for patients who received rifabutin (hazard ratios, 2.1 and 2.3), but there was no difference in survival (74, 181). A CD4 count of <100 per mm^3 at entry was the single most important determinant of progression to bacteremia in the placebo group; bacteremia occurred in 17.9% of those with CD4 counts of <50 per mm^3 and in 13.5% of those with CD4 counts of 50 to 99 per mm^3 but in only 5.6% of those with an initial CD4 count of 100 to 200 per mm^3 (234).

Prophylaxis with rifabutin also reduced the frequency of clinical signs and symptoms associated with MAC infection, such as fatigue, fever, anemia, and elevated alkaline phosphatase, and reduced the numbers of patients with Karnofsky scores of ≤70. There was, however, no difference in the incidence of chills, sweats, diarrhea, abdominal pain, or weight loss. The number of hospitalizations and the days of hospitalization were also reduced in patients who received rifabutin compared with those who received placebo (292 versus 373 total hospitalizations, respectively). Further analysis indicated that these clinical benefits were almost entirely limited to those with a $CD4^+$ cell count of <75 per mm^3 at entry to the study.

A sample of 88 MAC isolates from the two groups of patients was found to have susceptibility profiles similar to those of rifabutin and rifampin, suggesting that long-term use of rifabutin does not result in resistance to rifamycins. Only six patients developed tuberculosis during one of the studies; three patients who received placebo developed active tuberculosis and three patients who received rifabutin developed presumptive evidence of tuberculosis (not confirmed by culture). On the basis of this and other epidemiologic data, a U.S. Public Health Service Task Force recently recommended that prophylaxis be considered for all HIV-infected patients with fewer than 100 $CD4^+$ cells per mm^3 (461). Whether patients with evidence of colonization and $CD4^+$ cell counts of >100 per mm^3 would benefit from prophylaxis is not known.

LABORATORY DIAGNOSIS

Isolation and Identification

Several methods can be used to culture MAC from blood, bone marrow, or other specimens (5, 171, 280, 414), and the most sensitive laboratory diagnosis requires the use of at least two culture systems including both solid and liquid media (310). Contaminated specimens, such as respiratory and stool specimens, are processed to eliminate rapidly growing bacteria and yeasts prior to culture. With the radiometric BACTEC method, a sterile tissue specimen, blood (concentrated or unconcentrated), or a decontaminated specimen is inoculated into a modified Middlebrook 7H9 liquid medium containing radioactively labelled palmitic acid (BACTEC TB System; Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) (218, 280, 286). The presence of mycobacteria is indicated by the production of ^{14}C-labelled carbon dioxide usually in 7 to 14 days. Alternatively, a larger volume (up to 5 ml) of unconcentrated blood is inoculated directly into BACTEC 13A medium (281, 443), a medium developed specifically for diagnosing mycobacteremia. There is one report that MAC grows in the BACTEC 6A medium which is used with the nonradioactive BACTEC 660 blood culture system (346). However, use of this system requires performing an acid-fast smear on Gram stain-negative bottles, and *M. tuberculosis* does not grow in this medium.

Specimens also should be inoculated onto an agar-based medium such as Middlebrook 7H11 or an egg-based medium such as Lowenstein-Jensen, using a conventional method (280) or the new biphasic Septi-Chek AFB System (Becton Dickinson Microbiology Systems, Cockeysville, Md.). In two reports, the Septi-Chek AFB System proved as sensitive or more sensitive than either conventional culture or the BACTEC radiometric system (119, 254). The isolation of MAC and certain other NTM on egg-based media may be improved by lowering the pH to less than 6.5 and adding pyruvate or glycerol (269). Agy et al. (5) compared four blood culture systems for mycobacteria and found that the single most sensitive (94% of 32 positive cultures) system was the BACTEC 13A medium inoculated with 5 ml of blood collected in a Vacutainer tube containing sodium polyanethol sulfonate. The mean time to detection with either the BACTEC 13A or the BACTEC 12B was approximately 14 days, whereas the mean times to detection with Middlebrook 7H11 and a biphasic system were 21 and 24 days, respectively.

M. avium, like *M. tuberculosis*, is an intracellular pathogen, and organisms found in blood are most likely to be present in the circulating monocytes. Therefore, lysis of the blood cells by a commercial lysis-centrifugation method

(Isolator; Wampole Laboratories, Cranbury, N.J.) or treatment of the blood with a lytic agent such as sodium deoxycholate significantly improves the detection of mycobacteria in blood (171, 280, 504). Using blood inoculated with known concentrations of mycobacteria, von Reyn et al. (464) demonstrated that MAC isolates remain viable in Isolator tubes for at least 7 days.

Quantitative plate counts (CFU per milliliter) were used to monitor the efficacy of therapy in a variety of clinical trials, but the technique is laborious (the potentially high mycobacteremia requires several serial dilutions of the blood) and probably should be limited to investigative studies. The BACTEC system may provide a convenient and reliable approximation of the level of the bacteremia, since there appears to be a good correlation between the rate of growth (days to positive blood culture) in the BACTEC 13A system and the degree of bacteremia as determined by lysis-centrifugation (201). BACTEC blood cultures that were positive in less than 7 days had >400 CFU/ml, whereas those that were positive at ≥12 days had low levels of bacteremia (<9 CFU/ml).

MAC colonies tend to be visible sooner and colony morphology is more apparent on agar-based media, while the egg-based media promote pigment production. In general, positive cultures are detected within 7 to 14 days with the radiometric method, 21 to 28 days (or longer) by the conventional method (286), and approximately 20 days with the SeptiChek AFB System (254). At present, the most sensitive system would be a combination of the BACTEC radiometric system, using either 12B or 13A medium, and a 7H10 or 7H11 plate. Positive cultures should be confirmed by performing an acid-fast stain on the broth culture or plate-isolated microorganism. At that point, an experienced mycobacteriologist may be willing to report a presumptive identification on the basis of growth characteristics and microscopic morphology.

The identification of clinically significant mycobacteria can be achieved within a few hours, once sufficient growth is available, using nonradioactively labelled DNA probes (134, 178, 304, 383, 425). Positive broth cultures can be concentrated by centrifugation and directly tested with the probes; some investigators believe the results should be confirmed by testing organisms isolated on solid media (142, 380). The DNA probe tests are considered highly specific and sensitive. In one evaluation of the probe identification method, 114 MAC isolates were tested with the three available probes and results were compared with conventional identification (349). Initial results yielded a sensitivity of 93% and a specificity of 97%, but on repeat testing the sensitivity and specificity increased to 97 and 100%, respectively. A more recent evaluation of the acridinium ester-labeled probes from GenProbe (San Diego, Calif.), using 40 MAC isolates, showed 100% specificity and 95.2% sensitivity (294). Lim and coworkers (304) found that the SNAP (Syngene; San Diego, Calif.) oligonucleotide probes were more sensitive than the GenProbe probes primarily because seven isolates that were negative by GenProbe assay were positive with the Syngene X-probe (SNAP oligonucleotide probes are no longer commercially available). The acridinium ester-labeled probe for the MAC made by GenProbe was redesigned, and in a preliminary study it had a sensitivity of 99.5%; the false-negative isolates had unusual characteristics and may represent a previously unrecognized taxonomic branch of the complex (263). The probe used in the last study is now commercially available and has replaced the previously available MAC probe. By first testing acid-fast isolates from AIDS patients with the MAC oligonucleotide probe or highly characteristic colonies with the *M. tuberculosis* probe, the high cost of these probes can be controlled (134, 178). Conventional biochemical methods of identification can be improved by using a strategy that limits the number of tests (471).

By combining the radiometric method of detection and the DNA probe method of identification, a definitive laboratory diagnosis of disseminated MAC infection should take no longer than 4 weeks; however, it is not unreasonable for a clinician to expect a reliable detection and presumptive identification of disseminated MAC infection within 7 to 14 days. Peterson et al. (380) showed that by combining the BACTEC radiometric method of detection with radioactive GenProbe DNA probe identification, >86% of clinically significant MAC-positive specimens could be reported within 7 days. Recently, the same group evaluated the acridinium ester-labeled probes for *M. tuberculosis* and the MAC and found that MAC-positive BACTEC cultures were detected with a sensitivity and specificity of 99 and 96%, respectively (146). However, the presence of blood strongly interfered with the chemiluminescent reading of the hybridization assay, and it was necessary to treat samples from the BACTEC medium with detergent and EDTA prior to testing as well as to include a background control sample.

Direct Detection by Staining

The direct examination of blood lymphocytes (buffy coat blood film) stained with an acid-fast stain or negatively stained with Wright's or Romanovsky stain may reveal mycobacteria (143, 175, 362). Also, auramine-rhodamine staining with fluorescent microscopy of bone marrow aspirations may be of value (460); however, the positive predictive value of these techniques is variable (35 to 86%), and one cannot exclude *M. tuberculosis* bacteremia on the basis of a smear alone. Furthermore, mixed infections can occur, and clinicians and laboratorians should be alert to the possibility that the presence of MAC organisms on the culture plate may obscure the detection of *M. tuberculosis* (143) or coinfection with *M. simiae* (453). Wiley et al. (478) reported on the use of mixed polyclonal antibodies to three species of mycobacteria to detect microorganisms in tissue including bone marrow biopsies (Fig. 5). In this study, 32 of 34 cases of proven mycobacterial disease were positive by the immunohistochemical technique. In addition, 8 of 10 specimens that were negative by conventional staining methods were positive with the mixed-antibodies method. Using the same technique, Perry et al. (379) showed that the immunohistochemical stain was more sensitive than a Kinyoun stain, 90 versus 66%, with bone marrow core biopsies, but neither of these methods was as sensitive as blood culture. Furthermore, there was a poor correlation between the level of mycobacteremia and the number of bacilli observed by the immunohistochemical stain. Finally, there is a preliminary report (431) on the detection of MAC antigenuria which showed a low (<60%) sensitivity but good specificity (100%); however, the number of specimens tested was small.

PCR

The need for improved detection and identification of mycobacteria in clinical specimens has led to intensive interest in the application of this technology to this long-standing diagnostic need. Hance et al. (195) described an

amplification assay based on a 383-bp target within the highly conserved gene for the 65-kDa mycobacterial cell surface antigen. Species identification was achieved with probes for regions of the amplified target specific for *M. tuberculosis*, *M. avium*, or *M. fortuitum*. The sensitivity of the assay for *M. bovis* was 10 to 1,000 bacilli depending on the method of visualization. Ghossein et al. (169) recently applied the same PCR assay to the detection and species identification of mycobacteria in paraffin-embedded tissues. Tissue sections were deparaffinized and digested with proteinase K and boiled. Formalin-treated tissue that was 7 years old contained amplifiable DNA, and 11 of 12 tissues with acid-fast bacilli were positive in the PCR assay. Species identity was determined by restriction analysis, using *Nar*I, which generates restriction products that readily distinguish *M. tuberculosis* and the MAC. B5-fixed tissue failed to yield amplifiable DNA despite the presence of numerous acid-fast bacilli.

Boddinghaus et al. (60) described a generic mycobacterial PCR assay based on a target within a 16S rRNA sequence. Species identification was based on the hybridization of species-specific probes to variable regions within the amplified target. The assay detected as few as 10 *M. tuberculosis* bacilli; however, the performance of the assay was not assessed with clinical specimens containing MAC organisms. Although rRNA has certain advantages as a PCR target (rRNA is comparatively abundant and readily sequenced), the assay requires an additional reverse transcription step.

Plikaytis et al. (386) used a different approach and identified a large 1,380-bp target within the highly conserved *hsp65* gene which is present in at least 19 different species of mycobacteria. Species identification was based on RFLP profiles generated by endonuclease digestion of the PCR product with *Bst*NI or *Xho*I. The validity of the RFLP identities was assessed by using a computer program to normalize and cluster the various profiles. The complexity of the RFLP analysis would restrict the utility of this method, and in our experience amplification of the nearly 1,400-bp target was difficult to reproduce.

Soini et al. described a PCR assay that used a 423-bp target within the gene that encodes a common 32-kDa secretory antigen (434a). The target was amplified in 28 different species of mycobacteria, and a 21-mer probe hybridized to all *M. tuberculosis* amplicons but cross-reacted with several other species of mycobacteria. In several instances the assay was inhibited by unknown factors in the specimens (sputa). The inhibition of PCR assays by components of clinical specimens, including and perhaps especially blood, is common (333, 375), and erythrocyte lysis, phenol-chloroform extraction, or the use of a chelating resin is necessary to avoid inhibition by blood components (129).

Kunze et al. (290) described four separate PCR assays for targets within insertion sequences found in certain strains of MAC. RFLP analysis of the MAC showed that a certain RFLP type designated A commonly infects patients with AIDS; however, these strains do not contain insertion sequences. In contrast, all strains of *M. paratuberculosis* contain an insertion sequence, IS*900*, while another RFLP type designated A/I contains IS*901*, which is very similar to IS*900*. IS*901* has been associated with virulence in BALB/c mice. The four PCR assays were specific for (i) a 1,108-bp target within IS*901* found in RFLP type A/I strains; (ii) a 300-bp target which is up- and downstream of IS*901*, but also amplifies a 1,742-bp target in RFLP type A/I; (iii) a 453-bp target within IS*900*; and (iv) a 574- or 577-bp target that was common to both IS*900* and IS*901*. PCR analysis showed that none of the MAC strains from AIDS patients contained IS*900* or IS*901* while all but one isolate from birds contained IS*901* and 89% of *M. paratuberculosis* strains contained IS*900*. Thus, the 300-bp PCR product is defined by primer sequences within the flanking regions of IS*901*. The study did not address the potential use of these PCR assays to detect MAC in clinical specimens. Finally, Haas et al. (192) described preliminary results of a nested PCR assay for 533- and 75-bp targets within the gene for the Avi-3 antigen of *M. avium*.

In summary, although PCR detection and identification of MAC has considerable promise, no PCR assay has emerged with clear and compelling diagnostic utility that is based on an evaluation of a large number and variety of clinical specimens and correlation with a standard method. Recently, PCR assays for mycobacteria have become commercially available through Smith-Kline and Roche Diagnostic Laboratories; however, the performance characteristics of these tests with MAC-containing specimens and appropriate controls are unknown to us.

Diagnosis of MAC Disease in Patients with AIDS

The laboratory diagnosis of MAC infection in AIDS patients should be focused on the diagnosis of disseminated disease. The detection or isolation of MAC organisms from stools, duodenal biopsy or other gastrointestinal tract specimens, or bronchial washings or other respiratory tract specimens should be interpreted with caution. Certainly the repeated isolation from potentially colonized sites will facilitate assessment of the clinical significance of such findings. Although the isolation of MAC from stool or respiratory tract specimens clearly may presage the development of disseminated disease, the negative predictive value (cultures that fail to grow MAC) is poor (87, 198, 232). Neither skin test sensitivity (PPD-B or Battey antigen) nor serological assessment of anti-MAC antibodies by enzyme immunoassay (which is not readily available) appears to be of clinical use (295, 472, 475, 481); however, some evidence suggests that certain MAC antigens may have more diagnostic utility than others (30, 348).

The MAC may be cultured from a variety of normally sterile sites, including bone marrow, liver, spleen, and lymph nodes, and cultures should be done when these tissues are available, but blood culture is convenient, relatively noninvasive, and in most circumstances the procedure of choice for the diagnosis of disseminated MAC disease (150, 218, 280, 388). Yagupsky and Menegus (489), using the Isolator system, concluded that two blood cultures are sufficient to detect MAC bacteremia, and in their experience nearly 20% of these cultures contained only 1 CFU/ml.

IN VITRO SUSCEPTIBILITY TESTING

Background

In vitro susceptibility testing of MAC with the methods and interpretive criteria described for *M. tuberculosis* has little value in guiding the treatment of patients with MAC disease, by which we mean using the agar-based, modified-proportion method and critical concentrations of traditional antituberculous agents (244). There have been no controlled prospective clinical trials specifically designed to correlate in vitro susceptibility test results with clinical efficacy of MAC treatment regimens. In addition, the results of in vitro

methods used for testing NTM are difficult to interpret because the interlaboratory reproducibility is unknown and the methods lack standardization. The lack of antimicrobial agents with potent activity against the MAC (certainly no agent is as potent against the MAC as INH is against *M. tuberculosis*) has only confounded the situation further.

Despite these rather substantial limitations, in vitro susceptibility testing of the MAC is performed in many mycobacteriology laboratories, and some clinicians, usually those with extensive experience in treating these infections, find the results useful in guiding therapy. In recent years there has been increased interest in identifying reliable in vitro susceptibility test methods. Heifets (203) has persuasively argued that the most useful in vitro susceptibility test results are the quantitative MIC and MBC expressed in micrograms per milliliter. The accurate and precise measurement of bactericidal activity, especially the bactericidal activity of combinations of agents, ultimately may provide the most useful information for predicting the clinical efficacy of a particular treatment regimen. Nevertheless, definitive interpretive criteria will be difficult, if not impossible, to establish in the absence of controlled clinical trials with single and multiple agents.

The concentration of drugs in serum can be a useful criterion, but consideration also should be given to using tissue and phagocyte concentrations (when such information is known) as alternative interpretive criteria. It must be emphasized that there is little consensus on either the laboratory or the clinical definitions of susceptibility and resistance. In general, MAC isolates are predictably resistant to isoniazid and pyrazinamide and only variably susceptible to rifampin and ethambutol on the basis of studies that used a variety of susceptibility testing methods. The susceptibility patterns of MAC isolates appear to be considerably more variable than those of *M. tuberculosis* (203, 205, 249, 455), emphasizing the potential importance of susceptibility testing, especially as more antimycobacterial agents are identified with activity against the complex. There is some evidence that MAC isolates from AIDS patients are more resistant to antimicrobial agents than isolates from non-AIDS patients (227), but these results have been challenged by other studies (206).

Susceptibility testing of the MAC is complicated by the variations in colony morphology, since the translucent colony type is more resistant to antimicrobial agents than the opaque colony type (486). Also, Stormer and Falkinham (442) showed that nonpigmented variants of *M. avium* are significantly more resistant to a variety of antimicrobial agents than are pigmented segregants of the same strains. Since the pigmented variants grow more rapidly and are more obvious, Stormer and Falkinham (442) speculated that in the selection of *M. avium* colonies for susceptibility testing the less obvious nonpigmented variants, which may be analogous to the transparent colony variants, could be easily overlooked. Thus, testing only pigmented variants could lead to a false-susceptible result.

Both broth and agar dilution methods that measure, quantitatively, the in vitro activity of conventional and investigational antimycobacterial agents against the MAC have been described. Inderlied et al. (249) tested 56 strains of MAC by both agar and broth susceptibility test methods and found significant differences in the results. The MIC_{50}s and MIC_{90}s of ethambutol, ethionamide, and amikacin were two- to eightfold higher by the agar method; the agar method was a variation of the National Committee for Clinical Laboratory Standards agar dilution procedure with 7H11 agar supplemented with OADC substituted for Mueller-Hinton agar. In the same study, there was good agreement between agar- and broth-based results for isoniazid, streptomycin, and rifampin. Others have reported on the effect of media on susceptibility results (203, 204) and noted, in particular, that testing of ethambutol in an agar medium is problematic (205). Broth media should not contain Tween 80 or similar fatty acids that are known to disrupt the cell wall and membrane of mycobacteria and increase the activity of antimycobacterial agents, potentially causing the isolate to appear falsely susceptible (351, 411).

Susceptibility Testing Methods

Although there are no clearly compelling reasons to use broth media in preference to agar media for in vitro susceptibility testing of the MAC, largely because the lack of reliable interpretive criteria precludes a comparative assessment of the accuracy and precision of various methods, Heifets (204) identified three important advantages of a broth medium: (i) several antimycobacterial agents appear to irreversibly bind to agar or the protein components within an agar matrix; (ii) agar medium tests require longer incubation times to reach a discernible endpoint, which increases the potential for the degradation of unstable agents; and (iii) mycobactericidal agents appear, for reasons that are unexplained, to be less potent in agar, and regrowth may occur over the extended incubation times used with these media. On the basis of our experience and that of others, we concluded that in vitro susceptibility test methods that use a broth medium will more likely produce accurate and precise results than methods that use an agar medium (244).

Inderlied et al. (249) described a method that uses BACTEC 12B medium, broth which is useful for testing MAC against investigational agents. The controls for this method include no drug, inoculum diluted 1:100, and inoculum diluted 1:1,000. The 1:100 control follows the convention of measuring the activity of an antimycobacterial agent, using a breakpoint of 99% inhibition, while the 1:1,000 control more closely follows the convention used with rapidly growing aerobic bacteria (the endpoint of agar dilution is the absence of growth with a starting inoculum of 10^4 CFU, interpreted here as $\leq$10 CFU), and 10 CFU is the limit of sensitivity for the BACTEC method. The MIC of an antimycobacterial agent is measured by interpolation from a dose (micrograms per milliliter)-response curve at the point of intersection with the controls. The advantage of this method is that the MIC is a discrete value (as opposed to a doubling dilution) and information about the mode of action or the mechanism of resistance may be deduced from the kinetics of inhibition.

Heifets and Iseman (208) described a broth dilution method that used BACTEC 12B media and compared the daily change in CFU per milliliter with the growth index (GI) values. The controls included no drug and inoculum diluted 1:100. The MIC was defined as the lowest concentration of drug that inhibited an increase in the GI for at least 4 to 5 days compared with the control. In a later publication, Heifets (203) modified the protocol slightly by specifying that the GI control was >10 for 3 days. There was excellent correlation between the daily CFU per milliliter and the GI values for both the control and drug-treated cells.

Heifets and Iseman (204, 209) proposed criteria for interpreting in vitro results based on the serum concentration of the drug and the comparative activity of the drug with a susceptible strain of *M. tuberculosis*. According to these criteria, a MAC isolate would be considered susceptible or

moderately susceptible when the MIC of a drug was $<C_{max}$ for the drug and ≤ or ≥ the MIC for the susceptible strain of *M. tuberculosis*, respectively. A major reservation about these criteria is that the definition of susceptible for *M. tuberculosis* is based on the 99% endpoint, which may not be sufficiently rigorous for the MAC. This concern is emphasized by recent observations showing that the MAC develops resistance during monotherapy (80, 121) and that the mechanism(s) of resistance is unknown.

Yajko et al. (491) described a broth microdilution assay that used 7HSF broth (7H9 broth supplemented with casein hydrolysate, OADC, and glycerol) and commercially prepared microdilution test panels (Sceptor) to test 30 antimicrobial agents against 31 strains of the MAC isolated from patients with AIDS. In an earlier publication, Yajko et al. (492) described a broth macrodilution (2 ml per tube) assay that used unmodified 7H9 broth in conjunction with studies on the mycobactericidal activity of amikacin, rifabutin, ciprofloxacin, and clofazimine. With each method, the inocula were prepared with an overnight subculture from a 7-day primary culture in 7H9 broth. The source of the original inoculum was growth from a Lowenstein-Jensen slant, and the inocula were not subcultured to an agar medium to examine for colony variants. Endpoints for both the microdilution and the macrodilution methods were visible growth (turbidity). In these studies the MAC appeared susceptible to a variety of β-lactams and aminoglycosides at concentrations that can be achieved in serum. All strains grew luxuriantly in the 7H9 and 7HSF media, and endpoints were discernible within 7 days.

In vitro susceptibility testing of the new macrolides, clarithromycin and azithromycin, against the MAC has taken on greater significance with the recent reports that showed that these drugs are effective as single agents for the treatment of disseminated MAC disease (Table 3). Interpretive criteria for defining macrolide resistance are being developed; however, a two- or threefold dilution increase in the MIC for a MAC strain is likely to indicate resistance. For clarithromycin, a MIC of >16 μg/ml should be considered resistant; however, the in vitro testing of macrolides is strongly influenced by test parameters, notably, pH. Macrolides are significantly more active at pH 7.2 to 7.4 than at pH 6.8 to 6.9, which is a conventional pH for testing mycobacteria. Although raising the pH clearly improves the activity of macrolides, many strains of MAC may grow more slowly at this pH and one must be concerned that the inhibition of growth is a result of the combined effect of pH and the antimicrobial agent.

Testing Combinations of Drugs

The treatment of MAC pulmonary infections is predicted largely on the assumption that multiple antimycobacterial agents act in a synergistic manner. There are some in vitro and in vivo results that support this premise; however, the evidence is not compelling and certainly is contradicted by the experience with disseminated MAC infection in patients with AIDS (248, 502). Zimmer et al. (507) showed that 96% of 49 MAC isolates from patients with pulmonary disease were susceptible to serum concentrations of rifampin and ethambutol and this combination was synergistic. Synergistic activity was defined as a fourfold decrease in the MIC of the combination compared with the MIC of each agent alone. Heifets (207) examined two- and three-drug combinations of rifampin, ethambutol, ethionamide, and streptomycin tested against the MAC by using an isobologram analysis of subinhibitory concentrations. With this agar-based method, all two-drug combinations appeared to be synergistic against the three serovar 8 strains included in this study. Hoffner et al. (222) confirmed the synergistic interaction between ethambutol and rifampin or ethambutol and streptomycin against the MAC with an entirely different broth-based method.

In vitro susceptibility tests of combinations of antimycobacterial agents against the MAC may be necessary to identify synergistic interactions or to prevent the emergence of resistance. However, as with the in vitro susceptibility testing of single antimycobacterial agents, there are no standard methods for testing agents in combination and there is no consensus on interpretive criteria. Indeed, there is no uniform definition of synergistic, antagonistic, or additive activity as applied to the testing of the MAC. Nevertheless, at least four methods can be envisioned for measuring the interaction between antimycobacterial agents, but the method used to measure the activity of a combination of drugs depends on the nature of the question being addressed (244). Thus, a maximum effect or time-kill curve method is more appropriate to determine whether there is a synergistic bactericidal interaction. If an MBC is the endpoint of the assay, an isobologram method can be used to measure synergistic bactericidal activity. A time-kill curve and an emergence of resistance analysis are similar; e.g., the time-kill curve analysis may readily reveal subpopulations of resistant organisms, but it can also provide hints about the mechanism of action of drugs. An emergence of resistance analysis would be specifically designed to measure the synergistic effect of combinations of agents on an already identified resistant subpopulation.

The application of interpretive criteria to each of these methods is not uniform. There is some broad appeal for a 99.9% endpoint in time-kill curve measurements (355), but in comparing combinations of agents, a time factor should be included in the criteria. Indeed, the rate of killing may have more clinical significance than the degree of killing (355). The clinical relevance of a 99% endpoint for measuring the emergence of resistance was well established in studies of *M. tuberculosis*; however, this endpoint has not been verified for any of the other slowly growing mycobacteria, including MAC. The limits of the maximum-effect type of analysis will be set in part by pharmacokinetic features of the component drugs. Criteria for distinguishing significant differences between combinations of agents may not be easily established. Criteria for the isobologram type of analysis have been elegantly defined in terms of the fractional inhibitory concentration (24, 25). Heifets et al. (210) adapted the fractional inhibitory concentration type of analysis to study combinations of antimycobacterial agents with the MAC. MICs were determined by the BACTEC broth dilution method, and results for combinations of two drugs were based on fractions of the MICs. For testing two drugs alone and in combination, over 20 BACTEC vials were used, including 2 vials for controls. By using MBC endpoints, an identical method of analysis yields the fractional bactericidal concentration. Hoffner et al. (222) also used the BACTEC system for measuring the activity of antimycobacterial agents alone and in combination, but they analyzed for synergistic effects directly from the GI readings. GI values were measured on day 4, and synergy was defined as $X \div Y$ (min) $< 1 \div Z$, where X is the GI at day 4 of the combination, Y is the lowest GI of the agents alone, and Z is the number of agents in the combination. When the ratio was <0.5 for two drugs, the combination was considered synergistic; a

ratio of 1 indicated no interaction, and a ratio of 2 indicated antagonism. Yajko et al. (490) used a broth macrodilution method to measure the synergism of combinations of two, three, and four antimycobacterial agents with MAC. In this case, fractional inhibitory concentration and fractional bactericidal concentration indices were determined by the method of Hallander et al. (193), which is essentially the same as described above, i.e., the ΣFIC is the sum of the indices for all drugs in the combination. For each strain tested, drugs were added at the respective MICs and at serial twofold dilutions of the MICs. To determine the fractional bactericidal concentration, the respective MBCs were summed in an identical manner; the MBC was based on 99% killing and designated the MBC (acid-fast bacilli) to distinguish this value from the more conventional MBC that is based on 99.9% killing.

Animal Models

Most in vivo animal studies of MAC disease have focused on murine test systems for the evaluation of potential therapies. Of these murine systems, the most frequently used system has been the beige mouse model of disseminated MAC disease (52, 160). There has been some debate about whether the beige mouse, infected intravenously with MAC, truly mimics disseminated MAC infection in patients with AIDS. The primary criticism is that the nature of the immunodeficiency in the beige mouse is inherited rather than acquired, such as with HIV infection. The nature of the immunodeficiency in beige mice has not been fully defined but is believed to be both a defect in NK and T-helper cell function (163). The beige mouse is considered the murine equivalent of the Chediack-Higashi syndrome in humans which is an aggregation of granulation and nuclear structure abnormalities of leukocytes. Clinical symptoms of the disease are hepatosplenomegaly, lymphadenopathy, anemia, thrombocytopenia, and susceptibility to bacterial infections which commonly results in death during early childhood. Beige mice are susceptible to other opportunistic infections seen in patients with AIDS such as *Pneumocystis carinii* and cytomegalovirus; however, natural infections are rare, and infection usually is a result of direct or deliberate exposure. In general, the mouse has proven to be a reliable animal test system for mycobacterial infections, and there are only a few reports on the use of other animals such as rats.

Lefford (296) discussed animal models for atypical mycobacterial infections and placed emphasis on the problem of intraspecies variation in the virulence of the MAC strain used in the models. This concern is consistent with the growing consensus that disseminated MAC disease is primarily caused by selected strains of *M. avium* (194). As a result, the beige mouse model of disseminated MAC disease should be viewed as a combination of both an appropriately susceptible animal and infection with a strain of MAC that is reliably associated with signs and symptoms of this disease including bacteremia, tissue infection, and mortality (246, 247). Others have reported on the use of cyclosporin-treated rats (71) and thymectomized C57 black mice intravenously infused with monoclonal antibody in order to selectively deplete $CD4^+$ T cells (159) as alternatives to the beige mouse model. These alternative models have attractive features, including the ability to modulate the immunodeficiency; however, these models are disadvantageous because of increased cost, inconvenience, and the need for manipulation of individual animals, which can be a source of experimental variation. The use of murine leukemia virus-infected mice (MAIDS mouse) is an attractive alternative model because of the greater similarity to AIDS and the possibility of testing immunotherapies (369). Finally, the nude mouse is another possibility, but these animals are expensive and require the use of protected environments. Ji and colleagues (261) recently compared beige mice, nude mice, and immunocompetent mice as models for MAC infection, using MAC 101, and concluded that beige and nude mice were equivalent in susceptibility to infection whereas immunocompetent mice were comparatively resistant to infection.

Current evidence suggests that the most likely route of MAC infection in patients with AIDS is the gastrointestinal tract (120). Thus, the beige mouse model of MAC disease, which entails direct inoculation of a large number of mycobacteria into the blood, may be inappropriate for studies of prophylaxis. We recently showed that beige mice can be infected by an oral route upon exposure to either 10^4 or 10^5 CFU on five occasions over 10 days (34). This modification of the beige mouse model will be particularly relevant for evaluating the efficacy of orally administered drugs, since animals infected by the oral route were found to have large numbers of mycobacteria in the gut and appendix, closely resembling gastrointestinal tract infections seen in patients with AIDS. Animals were infected with five MAC strains, and after 4 weeks, bacteremia was detected in 27% of animals infected with MAC 101, with a mortality of 12%. The CFU per gram of tissue were as follows: liver, $1.4 \pm 0.8 \times 10^6$; spleen, $2.1 \pm 0.6 \times 10^7$; and appendix, $2.0 \pm 0.7 \times 10^5$. After 8 weeks, 100% of the animals had evidence of disseminated disease, with MAC isolated from the blood of up to 70% of animals.

CONCLUSION

Twenty years ago few people would have predicted the current level of interest in the MAC, but HIV infection and AIDS have brought a focus to many etiologic agents of opportunistic infections that could not have been predicted prior to 1981. The knowledge and information about the biochemistry, physiology, genetics, and epidemiology of MAC as well as about the pathogenesis, immune response, and treatment of MAC disease will be permanent and continuing resources for future studies about this interesting and diverse group of microorganisms. This knowledge and information will provide a foundation for a better understanding of the role of the immune system in intracellular infections and the importance of newly described microbial virulence factors. Furthermore, these studies have already provided insight into the mechanisms of action and therapeutic utility of tissue-active anti-infective agents, the identification of antimicrobial agents with activity against intrinsically resistant microorganisms, and an overall better understanding of the biochemistry, genetics, and physiology of mycobacteria.

ACKNOWLEDGMENTS

Work summarized in this review was supported in part by grants and contracts AI 25140 (to C.B.I. and L.E.M.B.), AI 25146 (to C.B.I.), and AI 25769 (to L.E.M.B.) from the National Institutes of Health and R91-CC86SD (to C.A.K.) from the State of California Universitywide AIDS Research Program through the California Collaborative Treatment Group and the AIDS Community Research Consortium, Redwood City, Calif.

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